REMARKS/ARGUMENTS

Claim 47 has been amended to define the compound as in previous claim 53. Claim 47 has been further amended to make the preamble congruent with the last step of the claim as requested by the Examiner. Support is provided at e.g., p. 22, line 4. Support for new claim 63 is provided at e.g., p. 21, lines 12-14. Claims 50-53 and 56-59 are now redundant and have been cancelled. Claims 49 and 55 have been amended for improved clarity. Support is provided at e.g.. p. 4, lines 31-32. No amendment should be construed as acquiescence in any ground of rejection. After entry of this amendment, claims 47-49, 54, 55, are pending and 60-62 are withdrawn.

Claims 47-49, 53-55 and 59 are rejected as under 35 U.S.C. § 112, second paragraph as allegedly being indefinite

The claims are rejected as indefinite due to the alleged lack of congruency between the preamble of the claim and the body of the claim. Independent claim 47 has been amended to replace "assess" in the preamble with "detect." Thus, the rejection of 47 and the claims depending therefrom is moot.

Claims 47-49, 54, and 55 are rejected as under 35 U.S.C. § 112, first paragraph as allegedly failing to comply with the written description requirement

The claims are rejected as allegedly lacking adequate written description for compounds that would indiscriminately bind to CMV in the absence or the presence of a mutation in CMV, *i.e.*, compounds that would recognize CMV regardless of a mutation. The Examiner acknowledges that the specification teaches several compounds, which applicants assert, bind to CMV (*see* p. 4 of the office action). It is the Examiner's position that applicants' assertion is not substantiated by any evidence and that there is no indication that the disclosed compounds bind to CMV.

The Examiner's position raises two factual issues that applicants will address as a preliminary matter. First, the requirement for indiscriminately binding CMV and mutant forms thereof does not impose any particular difficulties in identifying suitable compounds for use in

the claimed methods. The claimed methods do not require that the compound bind every conceivable mutant form; rather it is sufficient that the compound bind a reasonable number of mutations. A compound binds to CMV via a binding site on a surface protein. Of the many mutations that can occur in the complex genome of CMV, which spans about 230 kb, only a small number will affect the site bound by any particular compound. Some mutations will not cause any amino acid change either because they occur in noncoding regions or because of codon degeneracy. Other mutations will occur in metabolic proteins encoded by the virus not present in the capsid. Still other mutations will occur in capsid proteins not containing the epitope. Only a small number of mutations causing amino acid changes in the binding site or sufficiently proximal thereto to change its conformation would be expected to affect binding of a particular compound. Such mutations are expected to be few in number compared with the total set of possible mutations in a genome of about 230 kb. Any compound that binds wildtype CMV is thus expected to a bind a substantial number of mutant forms of CMV as well. Accordingly, identifying compounds that indiscriminately bind CMV and mutants is no more difficult than identifying compounds that bind CMV itself.

WO 02/17900 (copy attached), the published form of PCT 01/27363 (incorporated by reference in the specification at p.18, line 26 and p. 22, lines 30-34) discloses that methiothepin and octocloethepin (copies of structure attached) , which are also disclosed in the present specification (see p. 21, lines 11-14), have capacity to bind CMV (see Example 3 and Fig. 2 of WO02/17900). The formula specified in previous claim 53 and now claim 47, referred to in the specification as IIa, is a relatively narrow generic formula encompassing nominally substituted methiothepin and octocloethepin. All compounds encompassed within the formula have a fused tricyclic structure in which two six-membered aromatic rings are fused to a central ring. The central ring serves to hold the aromatic rings in place and further has an attached nitrogen heterocycle (Nhet). Due to the retained aromatic rings and the retained nitrogen heterocycle, one would expect binding characteristics to be similar.

Although no longer relevant in view of claim cancellations, it is noted for the record that there is similar evidence that other compounds disclosed in the specification also bind CMV. WO 02/17969 (copy attached) is the published form of PCT 01/27269 (incorporated by

reference in the specification at p. 18, lines 27-29 and p. 22, lines 30-34) discloses that IBZM (3-Iodo-2-hydroxy-6-methoxy-N[(1-ethyl-2-pyrrolidinyl)methyl]-benzamide), which is also disclosed in the present application, has the capacity to bind CMV (see Fig. 1 of WO02/17969). The formulae recited in previous claims 50 and 51 and designated I and Ia in the specification are generic forms of IBZM. Both formulae have an aryl amide or benzamide moiety in which the nitrogen atom of the amide is substituted with a methylene group bearing a nitrogen heterocycle (of which pyrrolidine, a five-membered ring is representative for Ia). Without intending to be bound by theory, the steric and electronic attributes of IBZM are constant through formula I and Ia. In particular, the nitrogen heterocycle (Nhet or pyrrolidine) have a nitrogen atom capable of participating in hydrogen bonding at a receptor site. Similarly, the carbonyl moiety of the amide can also participate in electronic and H-bonding interactions at a receptor active site. Finally, the aromatic moiety (benzene or Ar) is capable of pi/pi stacking with, for example, a phenylalanine group at a receptor active site. In view of the steric and electronic makeup of the compounds, they are expected to provide similar binding activity to IBZM.

Lest the Examiner allege that incorporation by reference of PCT applications is improper for essential material, it is respectfully submitted that the material provided by the incorporated PCT's is not essential to practice of the invention. The present application describes the relevant compounds and their capacity to bind CMV. The PCT applications merely provide data confirming what should be presumed in the absence of contrary evidence, namely, that the description of the present application is correct. The data providing confirmation are not necessary to the practice of the claimed invention, and need not be included in the specification.

Having addressed the underlying factual issues raised by the Examiner, Applicants now turn to the legal requirements of written description. As noted by MPEP 2163 at p. 2100-1526, first column, second paragraph, the issue of written description "most typically...arise[s] in the context of determining whether new or amended claims are supported by the description of the invention in the application as filed, whether a claimed invention is entitled to the benefit of an earlier priority date or effective filing date under 35 USC 119, 120 or

365(c) or whether a specification provides support for a claim corresponding to a count in an interference" [citations omitted].

"[R]ejection of an original claim for lack of written description should be rare." MPEP 2163.03. There is a "strong presumption that an adequate written description of the claim invention is present when the application is filed." MPEP 2163 I.A. "In claims involving chemical materials, generic formulae usually indicate with specificity what the generic claims encompass one skilled in the art can distinguish such a formula from others and can identify many of the species that the claims encompass. Accordingly, such a formula is normally an adequate description of the claimed genus." MPEP 21263II. 3. Nevertheless, "the issue of written description may arise even for an original claim" if the "claimed invention as a whole require[s] an essential or critical feature which is not adequately described in the specification and which is not conventional in the art or known to one of ordinary skill in the art." Id.

The present claims are essentially the same as the originally filed claims, and no issues of new matter have been raised. The present case does not therefore fall under the typical ambit of written description addressed by MPEP 2163 at p. 2100-156, col. 1, second paragraph. Rather, the present case arises in the rare situation when the PTO has the burden of overcoming the "strong presumption" that the originally filed claims have adequate written description.

Here, it is respectfully submitted that the Examiner has not established omission of an essential or critical feature which is not adequately described in the specification and which is not conventional in the art or known to one of ordinary skill in the art. As discussed above, the Examiner's comments regarding indiscriminate binding between CMV and mutants do not impose any difficulties beyond identifying compounds that bind to CMV. The Examiner's concerns regarding lack of evidence that exemplified compounds and genera of compounds bind CMV is addressed in PCT applications incorporated by reference as discussed above. The Examiner's comments that the exemplified compounds and genera of compounds are not representative of all compounds encompassed by applicants' broadest claims is moot in view of claim cancellations.

Given that the present claims are directed to uses of compounds defined by a formula and that evidence has been provided that compounds within this formula have the

desired property of binding to CMV, it is respectfully submitted that the Examiner has not met the burden of showing that this is one of the rare cases in which an essential element not conventional in the art has been omitted such that the original claims lack written description.

For these reasons, withdrawal of the rejection is respectfully requested.

Claims 47-49, 53-55 and 59are rejected as under 35 U.S.C. § 112, first paragraph as allegedly lacking enablement

Claims 47-49, 53-55 and 59 remain rejected under 35 USC 112, first paragraph as allegedly lacking enablement. The Examiner raises a number of issues that will be addressed in turn.

The Examiner first alleges that the specification does not disclose compounds that indiscriminately bind to CMV and mutants thereof. This raises the same issue discussed above under written description. As applicants have explained, two cited PCTs provide evidence that exemplified compounds have capacity to bind CMV. Compounds that bind CMV are also expected to bind a reasonable number of mutants thereof. The disclosed genera are expected to have similar properties to the exemplified compounds for the reasons discussed above. Moreover, other compounds known in the art to bind CMV (e.g. antibodies to CMV) or identifiable using art known methods can also be used. That there may be some mutant forms of CMV that are not bound by a given compound is not inconsistent with enablement. Enablement does not require that generic claims function in every conceivable circumstance. *Atlas Powder Co. v. E.I. du Pont de Nemours & Co.* 224 USPQ 409 (Fed. Cir. 1984).

Next the Examiner alleges that the compounds may bind to pathogens other than CMV. This rejection is based on the alleged lack of evidence that the disclosed compounds bind to CMV, which has been addressed above. The additional allegation that the disclosed compounds may bind to pathogens other than CMV, and that such pathogens may be present in samples with such frequency as to result in an unacceptable level of false positives is merely speculation and insufficient to overcome the PTO's burden of proof.

Moreover, contrary to the Examiner's position, it would have been a routine matter for the artisan to distinguish between DNA from CMV and other pathogens at the DNA

level even if only a segment of DNA was analyzed. It is well known in the art that even relatively short segments of DNA are often unique to an organism. For example, there are 4^{20} different possible nucleic acids of 20 nucleotides, a number much larger than the number of bases in the human genome. Probes and primers typically used for analyzing mutations are of this order of length (i.e., about 20 nucleotides). Thus, an artisan could usually distinguish one virus from another without difficulty. Moreover, applicants reiterate that enablement does not require that the claimed methods function in every conceivable circumstance: the mere possibility that there might occasionally be confusion between CMV and some other pathogen is not inconsistent with enablement.

The Examiner has also alleged the specification does not describe how to compensate for the loss of blood when blood is continuously withdrawn. Applicants previously replied that if larger quantities of blood are withdrawn,, the application describes a system for returning the blood (see, e.g., page 15, line 23 to page 17, line 24). The Examiner now discounts these comments on the basis that the claims do not state a return of the withdrawn blood to the patient. Applicants disagree.

The purpose of the claims is for applicant to particularly point out and distinctly claim the subject matter which he or she regards as his or her invention. MPEP 608.01(k). The purpose of the specification is to provide a written description of the invention and the manner and process of making and using the same. MPEP 608.01. The claims are thus a concise statement of what applicant regards as the invention, and the specification a much more lengthy description of how to make and use it. In general it is not permissible, much less necessary, for the claims to produce the detailed and often lengthy teaching of the specification as to how to make and use the invention.

An exception can be found when the claims omit an element which applicants have described as necessary in the specification or other statements of record. MPEP 2164.08(c). However, such is not the case here. The claims as currently drafted include both small and large volumes of blood. The issue of returning blood to a patient is relevant, if at all, only when very large volumes of blood are removed. Accordingly, the claims can be practiced over much of their scope without returning blood to a patient. Thus, a step of returning blood to a patient is

not an essential feature of the claims as presently drafted, but rather an optional feature applicable only in particular circumstances. Such an optional feature belongs in a description of how to make and use the claimed invention provided in the specification, not in the claimed invention itself.

In the previous office action, the Examiner alleged that the specification did not describe how to extract CMV or CVM-infected cells from the compound in the collector. Applicants responded that it is not necessary to do this for a number of nucleic acid analysis methods (e.g. PCR), and that even if necessary, extraction could be achieved by competing the CMV or CMV-infected cells from the compound with a large excess of compound or other compound that binds US28 that binds CMV.

The Examiner now responds that applicant is arguing limitations not found in the claims as the claims do not recite a device for analyzing mutations. This remark is not understood because applicants did not refer to such a device. In any event, applicants' remarks were simply pointing out how to make and use the claimed invention. There is no reason that this information should be recited in the claims, as discussed above.

The Examiner also alleges that step (a) of the claimed invention is not open-ended and lacks an outlet for extraction. In response, it is assumed that the Examiner is referring to claim 48 in that this is the only pending claim to refer to a collector. Claim 48 recites "flowing the blood into or *through* a collector" (emphasis supplied) implying that the collector can be open-ended. Moreover, even if the collector lacked an outlet, extraction could be performed through an inlet after collection was finished.

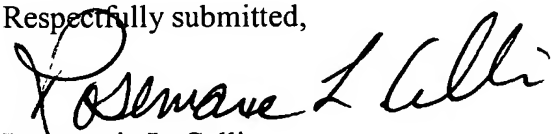
The Examiner next alleges that the claims lack enablement for an implant device in that the claims do not recite an outlet for retrieving CMV nor a retrieving of CMV from the implant. Initially, it is noted that only claim 54 refers to an implant device, and that insofar as relevant at all, this basis for rejection should only be directed to that claim. Even for this claim the Examiner has not provided the required showing that either feature is an essential element of the claim. MPEP 2172.01 provides that "essential elements" of the invention are those defined as such in the specification or from other statements of record. Here, the Examiner has simply provided an opinion that an outlet and retrieval of virus from the implant device are necessary

but has not pointed to any statements of applicants in the record or otherwise that support that position.

Finally, the Examiner alleges undue experimentation based on alleged lack of compounds that bind to CMV and mutations indiscriminately, lack of a working example, and lack of any teaching from the prior art concerning the use of compounds to capture CMV. Most of these issues have been addressed above. The disclosed compounds do bind to CMV as shown in PCT applications incorporated by reference. Compounds that bind wildtype CMV will also bind a reasonable number of mutant forms thereof. The presence or absence of a working example can be considered for enablement but is not determinative. Given the teaching of the specification regarding suitable compounds, the advanced state of the art in techniques such as analyzing mutations, and the high level of skill in the art of clinical diagnostics, it is respectfully submitted that full consideration of the relevant factors leads to a different conclusion. A skilled artisan would have been able to practice the claimed methods based on the teaching of the specification and knowledge in the art without undue experimentation.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 650-326-2400.

Respectfully submitted,



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RLC:aeb
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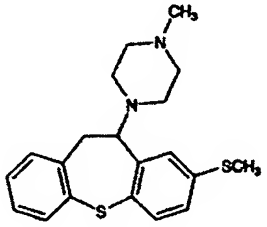
Merck Index, 12th Edition

6222. Metitepine. *1-[10,11-Dihydro-8-(methylthio)dibenzo[b,f]thiepin-10-yl]-4-methylpiperazine;* 8-methylthio-10-(4-methylpiperazino)-10,11-dihydrodibenzo[*b,f*]thiepine; methiothepine; methiothepin; Ro-8-6837. $C_{20}H_{24}N_2S_2$; mol wt 356.56. C 67.37%, H 6.78%, N 7.86%, S 17.99%. Serotonin ($5\text{-}HT_2$) receptor antagonist; also exhibits affinity for $5\text{-}HT_1$-receptors. Prepn: Neth. pat. Appl. 6,608,618; M. Protiva *et al.*, U.S. pat. 3,379,729 (1966, 1968 both to SPOFA); and pharmacology: K. Pelz *et al., Coll. Czech. Chem. Commun.* **33**, 1895 (1968); J. O. Jilek *et al., ibid.* **39**, 3338 (1974). Receptor-blocking study: M.-A. Monachon *et al., Arch. Pharmacol.* **274**, 192 (1972). Use in classification of 5-HT receptors: P. B. Bradley *et al., Neuropharmacology* **25**, 563 (1986); E. J. Mylecharane, *Clin. Exp. Pharmacol. Physiol.* **16**, 517 (1989).



Crystals from ethanol, mp 88-89°.

Maleate, $C_{20}H_{24}N_2S_2 \cdot C_4H_4O_4$, crystals from ethanol, mp 171-173°. LD_{50} in mice (mg/kg): 51 i.v.; 94 orally (Jilek).

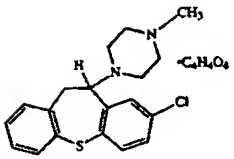
USE: Biochemical tool in serotonin receptor binding studies.

Sigma - RBI Catalog 1999

Octoclothepin maleate — 25 mg 29.00; 100 mg 85.00

D_2 Dopamine receptor antagonist; serotonin receptor antagonist.

1-(8-Chloro-10,11-dihydrodibenzo[b,f]thiepin-10-yl)-4-methyl-piperazine maleate
Mol. Wt. 460.98 $C_{19}H_{21}ClN_2S \cdot C_4H_4O_4$ [4789-68-8] Disposal A. White solid; mp 203-204°C. Store tightly sealed at RT. Slightly soluble in water or methanol. Solubility in 45% (w/v) aqueous 2-hydroxypropyl-β-cyclodextrin (Cat. No. H-107): > 21 mg/ml.



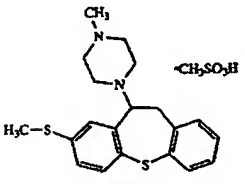
O-111

Hyttel et al. "Characterization of binding of ^{3}H-SCH 23390 to dopamine D_1 receptors. Correlation to other D-1 and D-2 measures and effect of selective lesions." *J. Neural Trans.* 68, 171 (1987); Nakajima et al. "[^{3}H]Ro 22-1319 (piquindone) binds to the D_2 dopaminergic receptor subtype in a sodium-dependent manner." *Mol. Pharmacol.* 26, 430 (1984); Wang Lu et al. "Effects of various neuroleptics, phenobarbital and SKF 525-A on dimethyltryptamine content in rat brain and liver." *Arch. Int. Pharmacodyn. Ther.* 232, 117 (1978).

Methiothepin mesylate — 100 mg 40.00; 250 mg 81.00
Metitepine mesylate

$5\text{-}HT_1$ Serotonin receptor antagonist; blocks serotonin autoreceptors.

1-[10,11-Dihydro-8-(methylthio)dibenzo[b,f]thiepin-10-yl]-4-methylpiperazine mesylate
Mol. Wt. 452.64 $C_{20}H_{24}N_2S_2 \cdot CH_3SO_3H$ [20229-30-5 (free base)] Disposal A. White solid; mp 188-190°C. Store tightly sealed at RT. Soluble in water (13 mg/ml).



M-149

Martin et al. "Comparison of the pharmacological characteristics of $5HT_1$ and $5HT_2$ binding sites with those of serotonin autoreceptors which modulate serotonin release." *Naunyn-Schmiedeberg's Arch. Pharmacol.* 321, 165-170 (1982); Nelson et al. "*In vitro* and *in vivo* disposition of [^{3}H]-Methiothepin in brain tissues." *Ibid* 310, 25-33 (1979); Pettibone et al. "Effects of methiothepin and lysergic acid diethylamide on serotonin release *in vitro* and serotonin synthesis *in vivo*: Possible relation to serotonin autoreceptor function." *J. Neurochem.* 43, 83-90 (1984).

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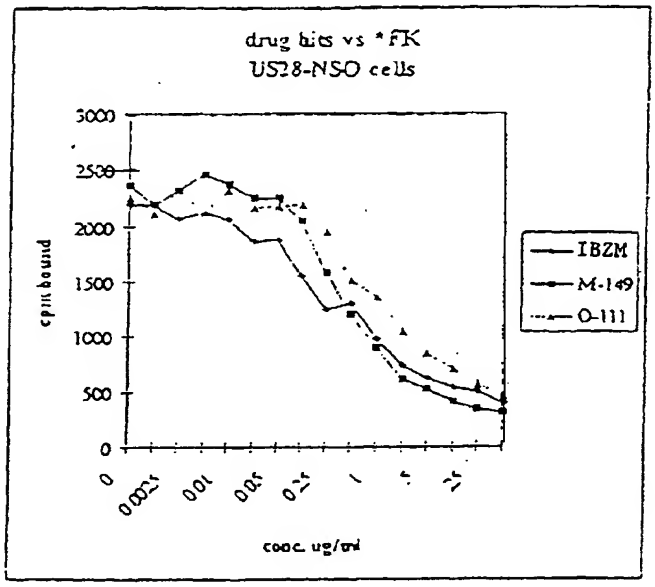
(54) Title: REAGENTS AND METHODS FOR THE DIAGNOSIS OF CMV DISSEMINATION



The IC_{50} values for each were: M-149, 0.3 μM; IBZM, 0.6 μM; and O-111, 0.7 μM.

(57) Abstract: Methods are provided for detecting the spread of cytomegalovirus in a host infected with CMV, by administering to the host a detectable and labeled amount of a non-endogenous compound which binds to US28 or a US28 fragment. Typically, the methods use a labeled form of IBZM.

WO 02/17969 A2

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

REAGENTS AND METHODS FOR THE DIAGNOSIS OF CMV DISSEMINATION

CROSS-REFERENCES TO RELATED APPLICATIONS

This application claims the benefit of US Provisional Patent Application Serial No. 60/229,191, filed August 30, 2000, the disclosure of which is incorporated herein by reference.

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

Not applicable

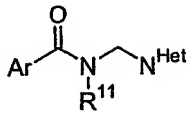
BACKGROUND OF THE INVENTION

Cytomegalovirus (CMV) is an important human pathogen and a major opportunist which emerges to cause disease in the immuno-compromised such as AIDS patients, neonates, and individuals who have been given immunosuppressive drugs as part of a transplantation regimen. In these individuals, the consequences of CMV in acute or re-emerging infections can be dire, including retinitis, encephalitis, and pneumocystis, among other pathologies. Furthermore, in immuno-competent hosts, CMV establishes a persistent lifelong infection through which it has been linked to a variety of inflammatory conditions including coronary artery occlusion following heart transplant and arthrectomy and restenosis following angioplasty. CMV interacts with leukocytes during acute infection of the host as well as during lifelong latency. As such, leukocytes are important players in CMV-induced disease and have been implicated in the acute phase of infection as vehicles for dissemination of virus and as sites of residence during lifelong latency.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides methods for detecting the spread of cytomegalovirus in a host infected with CMV, by administering to the host a detectable and labeled amount of a non-endogenous compound which binds to US28 or a

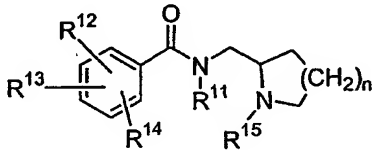
US28 fragment. Typically, the methods use a labeled form of a compound of the formula:



(I)

or a pharmaceutically acceptable salt thereof; wherein Ar represents a substituted aryl group; R^{11} represents H or (C_1-C_4)alkyl; and N^{Het} is a substituted or unsubstituted 4-, 5-, 6-, or 7-membered nitrogen heterocycle.

Preferred embodiments within this group are those compounds having the formula:



(Ia)

or a pharmaceutically acceptable salt thereof; wherein the subscript n is an integer of from 1 to 3; R^{11} and R^{15} are independently selected from H and (C_1-C_4)alkyl; R^{12}, R^{13} and R^{14} are each members independently selected from H, halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)haloalkyl, (C_1-C_4)haloalkoxy, nitro, cyano, (C_1-C_4)acyl, amino, (C_1-C_4)alkylamino, and di(C_1-C_4)alkylamino; with the proviso that at least one of R^{12}, R^{13} and R^{14} is other than H.

In certain preferred embodiments within this group, n is 1, R^{11} is H, R^{15} is (C_1-C_4)alkyl; and R^{12}, R^{13} and R^{14} are each other than H. In the most preferred embodiments, the compound is a labeled form of S(-)-3-Iodo-2-hydroxy-6-methoxy-N[(1-ethyl-2-pyrrolidinyl)methyl]-benzamide. A preferred labeled form is the [^{123}I]-labeled form.

In another aspect, the present invention provides methods for blocking CMV dissemination in a host by administering to the host an effective amount of a compound which blocks the binding of a chemokine to US28. Preferably, the compound is a compound represented by the formulae above. In this group of embodiments, the compound is preferably unlabeled.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the specific displacement of chemokine (fractalkine) binding to the US28 chemokine receptor by IBZM.

Figure 2 illustrates the Ca^{+2} flux profile between IBZM and a chemokine ligand (fractalkine) for US28.

Figure 3 illustrates the reversibility of IBZM binding to US28. In this figure, IBZM is pre-incubated with US28 expressing cells (at concentrations of 0-10 μg/mL) and removed by competition with fractalkine.

DETAILED DESCRIPTION OF THE INVENTION

Abbreviations and Definitions

Abbreviations: CMV, cytomegalovirus; S(-)-IBZM, S(-)-3-Iodo-2-hydroxy-6-methoxy-N[(1-ethyl-2-pyrrolidinyl)methyl]-benzamide.

The term "alkyl," by itself or as part of another substituent, means, unless otherwise stated, a straight or branched chain, or cyclic hydrocarbon radical, or combination thereof, which may be fully saturated, mono- or polyunsaturated and can include di- and multivalent radicals, having the number of carbon atoms designated (*i.e.* C_1-C_{10} means one to ten carbons). Examples of saturated hydrocarbon radicals include groups such as methyl, ethyl, n-propyl, isopropyl, n-butyl, t-butyl, isobutyl, sec-butyl, cyclohexyl, (cyclohexyl)methyl, cyclopropylmethyl, homologs and isomers of, for example, n-pentyl, n-hexyl, n-heptyl, n-octyl, and the like. An unsaturated alkyl group is one having one or more double bonds or triple bonds. Examples of unsaturated alkyl groups include vinyl, 2-propenyl, crotyl, 2-isopentenyl, 2-(butadienyl), 2,4-pentadienyl, 3-(1,4-pentadienyl), ethynyl, 1- and 3-propynyl, 3-butynyl, and the higher homologs and isomers. When used alone, the term "alkyl" refers to unsubstituted versions of the groups noted above. Groups provided as "substituted" are described in detail below.

The terms "alkoxy," "alkylamino" and "alkylthio" (or thioalkoxy) are used in their conventional sense, and refer to those alkyl groups attached to the remainder of the molecule via an oxygen atom, an amino group, or a sulfur atom, respectively.

The term "heteroalkyl," by itself or in combination with another term, means, unless otherwise stated, a stable straight or branched chain, or cyclic hydrocarbon radical, or combinations thereof, consisting of the stated number of carbon atoms and from one to three heteroatoms selected from the group consisting of O, N, Si and S, and wherein the nitrogen and sulfur atoms may optionally be oxidized and the nitrogen heteroatom may optionally be quaternized. The heteroatom(s) O, N and S may be placed at any interior position of the heteroalkyl group. The heteroatom Si may be placed at any position of the heteroalkyl group, including the position at which the alkyl group is attached to the remainder of the molecule. Examples include $-CH_2-CH_2-O-CH_3$, $-CH_2-CH_2-NH-CH_3$, $-CH_2-CH_2-N(CH_3)-CH_3$, $-CH_2-S-CH_2-CH_3$, $-CH_2-CH_2-S(O)-CH_3$, $-CH_2-CH_2-S(O)_2-CH_3$, $-CH=CH-O-CH_3$, $-Si(CH_3)_3$, $-CH_2-CH=N-OCH_3$, and $-CH=CH-N(CH_3)-CH_3$. Up to two heteroatoms may be consecutive, such as, for example, $-CH_2-NH-OCH_3$ and $-CH_2-O-Si(CH_3)_3$.

The terms "cycloalkyl" and "heterocycloalkyl", by themselves or in combination with other terms, represent, unless otherwise stated, cyclic versions of "alkyl" and "heteroalkyl", respectively. Additionally, for heterocycloalkyl, a heteroatom can occupy the position at which the heterocycle is attached to the remainder of the molecule. Examples of cycloalkyl include cyclopentyl, cyclohexyl, 1-cyclohexenyl, 3-cyclohexenyl, cycloheptyl, and the like. Examples of heterocycloalkyl or heterocyclyl include 1 -(1,2,5,6-tetrahydropyridyl), 1-piperidinyl, 2-piperidinyl, 3-piperidinyl, 4-morpholinyl, 3-morpholinyl, tetrahydrofuran-2-yl, tetrahydrofuran-3-yl, tetrahydrothien-2-yl, tetrahydrothien-3-yl, 1 -piperazinyl, 2-piperazinyl, and the like.

The terms "halo" or "halogen," by themselves or as part of another substituent, mean, unless otherwise stated, a fluorine, chlorine, bromine, or iodine atom. Additionally, terms such as "haloalkyl," are meant to include monohaloalkyl and polyhaloalkyl. For example, the term "(C_1-C_4)haloalkyl" is mean to include trifluoromethyl, 2,2,2-trifluoroethyl, 4-chlorobutyl, 3-bromopropyl, and the like.

The term "acyl" is used in its conventional sense and refers to an organic radical derived from an organic acid by the removal of the hydroxyl group. Examples of "acyl" groups include acetyl, propionyl, butanoyl, hexanoyl, isobutyryl, octanoyl, and the like.

The term "aryl" means, unless otherwise stated, a polyunsaturated, typically aromatic, hydrocarbon substituent which can be a single ring or multiple rings (up to three rings) which are fused together or linked covalently. The term "heteroaryl"

refers to aryl groups (or rings) that contain from one to four heteroatoms selected from N, O, and S, wherein the nitrogen and sulfur atoms are optionally oxidized, and the nitrogen atom(s) are optionally quaternized. A heteroaryl group can be attached to the remainder of the molecule through a heteroatom. Non-limiting examples of aryl and heteroaryl groups include phenyl, 1-naphthyl, 2-naphthyl, 4-biphenyl, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 3-pyrazolyl, 2-imidazolyl, 4-imidazolyl, pyrazinyl, 2-oxazolyl, 4-oxazolyl, 2-phenyl-4-oxazolyl, 5-oxazolyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl, 5-benzothiazolyl, purinyl, 2-benzimidazolyl, 5-indolyl, 1-isoquinolyl, 5-isoquinolyl, 2-quinoxalinyl, 5-quinoxalinyl, 3-quinolyl, and 6-quinolyl. Substituents for each of the above noted aryl and heteroaryl ring systems are selected from the group of acceptable substituents described below.

For brevity, the term "aryl" when used in combination with other terms (*e.g.*, aryloxy, arylthioxy, arylalkyl) includes both aryl and heteroaryl rings as defined above. Thus, the term "arylalkyl" is meant to include those radicals in which an aryl group is attached to an alkyl group (*e.g.*, benzyl, phenethyl, pyridylmethyl and the like) including those alkyl groups in which a carbon atom (*e.g.*, a methylene group) has been replaced by, for example, an oxygen atom (*e.g.*, phenoxymethyl, 2-pyridyloxymethyl, 3-(1-naphthyloxy)propyl, and the like).

Each of the above terms (*e.g.*, "alkyl," "heteroalkyl," "aryl" and "heteroaryl") when indicated as "substituted" can include a variety of substituents which provide a stable moiety. Preferred substituents for each type of radical are provided below.

Substituents for the alkyl and heteroalkyl radicals (including those groups often referred to as alkoxy, alkenyl, heteroalkenyl, alkynyl, cycloalkyl, heterocycloalkyl, cycloalkenyl, and heterocycloalkenyl) can be a variety of groups selected from: -OR', =O, =NR', =N-OR', -NR'R", -SR', -halogen, -SiR'R"R"', -OC(O)R', -C(O)R', -CO_2R', -CONR'R", -OC(O)NR'R", -NR"C(O)R', -NR'-C(O)NR"R"', -NR"$C(O)_2$R', -NH-$C(NH_2)$=NH, -NR'$C(NH_2)$=NH, -NH-$C(NH_2)$=NR', -S(O)R', -$S(O)_2$R', -$S(O)_2$NR'R", -CN and -NO_2 in a number ranging from zero to (2m'+1), where m' is the total number of carbon atoms in such radical. R', R" and R"' each independently refer to hydrogen, unsubstituted (C_1-C_8)alkyl and heteroalkyl, unsubstituted aryl, aryl substituted with 1-3 halogens, unsubstituted alkyl, alkoxy or thioalkoxy groups, or aryl-(C_1-C_4)alkyl groups. When R' and R" are attached to the same nitrogen atom, they can be combined with the

nitrogen atom to form a 5-, 6-, or 7-membered ring. For example, -NR'R" is meant to include 1-pyrrolidinyl and 4-morpholinyl. From the above discussion of substituents, one of skill in the art will understand that the term "alkyl" is meant to include groups such as haloalkyl (*e.g.*, $-CF_3$ and $-CH_2CF_3$) and acyl (*e.g.*, $-C(O)CH_3$, $-C(O)CF_3$, $-C(O)CH_2OCH_3$, and the like).

Substituents for the aryl and heteroaryl groups are varied and are selected from: -halogen, -OR', -OC(O)R', -NR'R", -SR', -R', -CN, $-NO_2$, $-CO_2R'$, -CONR'R", -C(O)R', -OC(O)NR'R", -NR"C(O)R', $-NR"C(O)_2R'$, ,-NR'-C(O)NR"R"', $-NH-C(NH_2)=NH$, $-NR'C(NH_2)=NH$, $-NH-C(NH_2)=NR'$, -S(O)R', $-S(O)_2R'$, $-S(O)_2NR'R"$, $-N_3$, $-CH(Ph)_2$, perfluoro(C_1-C_4)alkoxy, and perfluoro(C_1-C_4)alkyl, in a number ranging from zero to the total number of open valences on the aromatic ring system; and where R', R" and R"' are independently selected from hydrogen, (C_1-C_8)alkyl and heteroalkyl, unsubstituted aryl and heteroaryl, (unsubstituted aryl)-(C_1-C_4)alkyl, and (unsubstituted aryl)oxy-(C_1-C_4)alkyl.

Two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula $-T-C(O)-(CH_2)_q-U-$, wherein T and U are independently -NH-, -O-, $-CH_2-$ or a single bond, and q is an integer of from 0 to 2. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula $-A-(CH_2)_r-B-$, wherein A and B are independently $-CH_2-$, -O-, -NH-, -S-, -S(O)-, $-S(O)_2-$, $-S(O)_2NR'-$ or a single bond, and r is an integer of from 1 to 3. One of the single bonds of the new ring so formed may optionally be replaced with a double bond. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula $-(CH_2)_s-X-(CH_2)_t-$, where s and t are independently integers of from 0 to 3, and X is -O-, -NR'-, -S-, -S(O)-, $-S(O)_2-$, or $-S(O)_2NR'-$. The substituent R' in -NR'- and $-S(O)_2NR'-$ is selected from hydrogen or unsubstituted (C_1-C_6)alkyl.

As used herein, the term "heteroatom" is meant to include oxygen (O), nitrogen (N), sulfur (S) and silicon (Si).

The term "pharmaceutically acceptable salts" is meant to include salts of the active compounds which are prepared with relatively nontoxic acids or bases, depending on the particular substituents found on the compounds described herein. When compounds of the present invention contain relatively acidic functionalities, base addition salts can be obtained by contacting the neutral form of such compounds with a sufficient

amount of the desired base, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable base addition salts include sodium, potassium, calcium, ammonium, organic amino, or magnesium salt, or a similar salt. When compounds of the present invention contain relatively basic functionalities, acid addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired acid, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable acid addition salts include those derived from inorganic acids like hydrochloric, hydrobromic, nitric, carbonic, monohydrogencarbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulfuric, monohydrogensulfuric, hydriodic, or phosphorous acids and the like, as well as the salts derived from relatively nontoxic organic acids like acetic, propionic, isobutyric, maleic, malonic, benzoic, succinic, suberic, fumaric, mandelic, phthalic, benzenesulfonic, p-tolylsulfonic, citric, tartaric, methanesulfonic, and the like. Also included are salts of amino acids such as arginate and the like, and salts of organic acids like glucuronic or galactunoric acids and the like (see, for example, Berge, S.M., et al, "Pharmaceutical Salts", *Journal of Pharmaceutical Science*, **1977**, *66*, 1-19). Certain specific compounds of the present invention contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts.

The neutral forms of the compounds may be regenerated by contacting the salt with a base or acid and isolating the parent compound in the conventional manner. The parent form of the compound differs from the various salt forms in certain physical properties, such as solubility in polar solvents, but otherwise the salts are equivalent to the parent form of the compound for the purposes of the present invention.

In addition to salt forms, the present invention provides compounds which are in a prodrug form. Prodrugs of the compounds described herein are those compounds that readily undergo chemical changes under physiological conditions to provide the compounds of the present invention. Additionally, prodrugs can be converted to the compounds of the present invention by chemical or biochemical methods in an *ex vivo* environment. For example, prodrugs can be slowly converted to the compounds of the present invention when placed in a transdermal patch reservoir with a suitable enzyme or chemical reagent.

Certain compounds of the present invention can exist in unsolvated forms as well as solvated forms, including hydrated forms. In general, the solvated forms are equivalent to unsolvated forms and are intended to be encompassed within the scope of

the present invention. Certain compounds of the present invention may exist in multiple crystalline or amorphous forms. In general, all physical forms are equivalent for the uses contemplated by the present invention and are intended to be within the scope of the present invention.

Certain compounds of the present invention possess asymmetric carbon atoms (optical centers) or double bonds; the racemates, diastereomers, geometric isomers and individual isomers are all intended to be encompassed within the scope of the present invention.

The compounds of the present invention may also contain unnatural proportions of atomic isotopes at one or more of the atoms that constitute such compounds. For example, the compounds may be radiolabeled with radioactive isotopes, such as for example tritium (^{3}H), iodine-125 (^{125}I) or carbon-14 (^{14}C). All isotopic variations of the compounds of the present invention, whether radioactive or not, are intended to be encompassed within the scope of the present invention.

General

CMV harbors in its genome an open reading frame (ORF), designated US28, which encodes a protein that acts as a functional receptor for certain human and viral chemokines. Upon infection of a cell by CMV, US28 is expressed on the surface of the infected cell and becomes capable of responding to chemokines in the environment. Because the virus on its own is inherently non-motile, and because chemokines and their receptors encoded by human cells are known to regulate the migration of leukocytes and other cells through the body, CMV US28 is thought to be encoded by the virus to facilitate the dissemination of CMV through the body during and after infection. Therefore, agents which block the binding of chemokines to US28 should prove useful in inhibiting viral dissemination during acute or re-emerging CMV infection.

CMV US28 has been shown to bind a number of human, murine, and virus-encoded CC chemokines in a variety of assay formats. In addition, the CX3C chemokine, Fractalkine, binds with a very high affinity (K_I~50 pM) to US28. Fractalkine is expressed on certain endothelial cell surfaces and on populations of dendritic cells

(DC), and may thus define a portal through which CMV infected cells go from the circulation to the tissue space, as well as find residence in the DC.

Since the US28 receptor is expressed on cytomegalovirus infected cells, and also in view of its ability to bind multiple chemokines, a small molecule which specifically binds to this receptor would have significant use as an agent to diagnose the spread of CMV, and also as an anti-CMV agent.

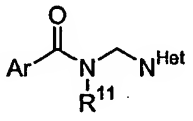
CMV US28 chemokine receptor is expressed on the surface of cells after infection by CMV. The receptor binds a number of chemokines and triggers viral dissemination. Accordingly, US28 (or fragments having chemokine binding activity) can be used to screen for inhibitors of chemokine binding to this receptor (see Co-pending Application Ser. No.60/229,365, Attorney Docket No. 019934-002500US, filed 08/30/00). Additionally, compounds which bind to US28 are useful for following the dissemination of the virus in a host. We have now discovered that S(-)-3-Iodo-2-hydroxy-6-methoxy-N[(1-ethyl-2-pyrrolidinyl)methyl]benzamide (S(-)-IBZM or IBZM, from the RBI division of Sigma-Aldrich) is an effective inhibitor of the binding of native chemokine ligands (such as fractalkine and eotaxin, among others), to the chemokine receptor encoded by the US28 open reading frame of human cytomegalovirus (CMV). Moreover, this compound was found to bind specifically to US28 among all chemokine receptors tested. Historically IBZM has been known to bind to D2 dopamine receptors in humans and other species. However, the compound has not been associated with any methods for the detection, diagnosis and imaging, or treatment of CMV. The chemical structure of IBZM includes an accessible iodide moiety suitable for substitution with the radiolabeled tracer ^{123}Iodine. [^{123}I]-IBZM has been used clinically in humans and other species for imaging of the distribution of D2 dopamine receptors by SPECT or PET scanning technologies. As a result of IZBM's specific chemokine receptor binding and its ready availability in a labeled form, the compound has particular utility for *in vivo* detection, diagnosis, and imaging of CMV infection. Unlabeled forms of IBZM and related derivatives also have utility for treatment of CMV dissemination by blocking chemokine binding to US28 on cell surfaces, an event which triggers viral dissemination.

Description of the Embodiments

A. Methods for detecting, diagnosing or imaging CMV infection in a host.

In one aspect, the present invention provides methods for diagnosing CMV in a host having CMV, the methods comprising:

(a) administering to the host an image-generating amount of a compound having the formula:



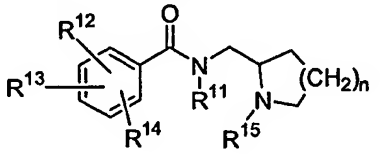
(I)

or a pharmaceutically acceptable salt thereof; wherein Ar represents a substituted aryl group; R^{11} represents H or $(C_1$-$C_4)$alkyl; and N^{Het} is a substituted or unsubstituted 4-, 5-, 6-, or 7-membered nitrogen heterocycle; and

(b) detecting sites at which the compound binds to US28 on cell surfaces present in the host.

In one group of preferred embodiments, Ar is a substituted phenyl group. In another group of preferred embodiments, Ar is a substituted phenyl group and N^{Het} is a substituted or unsubstituted pyrrolidinyl, substituted or unsubstituted piperidinyl, substituted or unsubstituted piperazinyl, substituted or unsubstituted piperidyl or a substituted or unsubstituted morpholinyl.

More preferably, the compound has the formula:



(Ia)

or a pharmaceutically acceptable salt thereof; wherein the subscript n is an integer of from 1 to 3; R^{11} and R^{15} are independently selected from H and substituted or unsubstituted $(C_1$-$C_4)$alkyl; R^{12}, R^{13} and R^{14} are each members independently selected from H, halogen, $(C_1$-$C_4)$alkyl, $(C_1$-$C_4)$alkoxy, $(C_1$-$C_4)$haloalkyl, $(C_1$-$C_4)$haloalkoxy, nitro, cyano, $(C_1$-$C_4)$acyl, amino, $(C_1$-$C_4)$alkylamino, and di$(C_1$-$C_4)$alkylamino; with the proviso that at least one of R^{12}, R^{13} and R^{14} is other than H.

In one group of embodiments, n is one; R^{11} is H; R^{12}, R^{13} and R^{14} are each independently selected from H, hydroxy, halogen, $(C_1$-$C_4)$alkyl and $(C_1$-$C_4)$alkoxy; and R^{15} is $(C_1$-$C_4)$alkyl.

A variety of labeled forms of the compounds described herein are available. For example, compounds of formula I or formula Ia in which the aryl group has a halogen substituent can be prepared using a suitable isotope of the halogen atom. Additionally, the labeled atom can be readily introduced in the penultimate synthesis step. For example, benzoic acid can be radioiodinated using conventional methods, then coupled to a suitable aminomethyl(heterocycle) to form the target compound useful for imaging. Alternatively, R^{11} or R^{15} can be a haloalkyl group which is incorporated into the structure in the final synthesis step.

The compounds of the invention therefore provide improved methods for imaging the CMV in a subject using PET and SPECT. The methods entail administering to a subject (which can be human or animal, for experimental and/or diagnostic purposes) an image-generating amount of a compound of the invention, labeled with the appropriate isotope and then measuring the distribution of the compound by PET if ^{18}F or another positron emitter is employed, or SPECT if ^{123}I or another gamma emitter is employed. An image-generating amount is that amount which is at least able to provide an image in a PET or SPECT scanner, taking into account the scanner's detection sensitivity and noise level, the age of the isotope, the body size of the subject and route of administration, all such variables being exemplary of those known and accounted for by calculations and measurements known to those skilled in the art without resort to undue experimentation.

Accordingly, one of R^{12}, R^{13} or R^{14} is preferably a halogen which can be prepared in a PET-labeled, SPECT-labeled or radiolabeled form. Particularly preferred halogen labels are ^{18}F, ^{75}Br, ^{123}I and ^{125}I. In the most preferred embodiments, one of R^{12}, R^{13} or R^{14} is iodine, and in labeled form is ^{123}I.

It is understood that compounds of the invention can be labeled with an isotope of any atom or combination of atoms in the structure. While ^{18}F, ^{75}Br, ^{123}I and ^{125}I have been emphasized herein as being particularly useful for PET, SPECT and tracer analysis, other uses are contemplated including those flowing from physiological or pharmacological properties of stable isotope homologs and is apparent to those skilled in the art.

The compounds of formulae I and Ia can be prepared using conventional synthetic methods known to those of skill in the art. In particular, compounds of formula Ia have been described in, for example, Schmidt, et al., *J. Pharm. Sci.* **88(3)**:305-315 (1994), and in references cited therein. Other compounds are described in PCT publication WO 95/04051, WO 90/09170, U.S. Patent No. 5,190,741 and EP 320630.

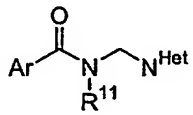
Imaging methods useful with labeled forms of IBZM other compounds of formula I and Ia have been described in, for example, Singhaniyom, et al., *Brian Res.* **453(1-2)**:393-6 (1988); Kung, et al., *J. Nucl. Med.* **31(5)**:573-9 (1990); Verhoeff, et al., *Int. J. Rad. Appl. Instrum. B.* **18(8)**:837-46 (1991); John, et al., *J. Nuc. Med.* **34(12)**:2169-75 (1993); Berding, et al., *Nuklearmedizin.* **33(5)**:194-9 (1994); Brandau, et al., *J. Nucl. Med.* **37(11)**:1865-71 (1996); Dence, et al., *Nucl. Med. Biol.* **24(4)**:333-40 (1997); Kufferle, et al., *Psychopharmacology (Berl).* **133(4)**:323-8 (1997); Zamora, et al., *Life Sci.* **63(18)**:1611-8 (1998); Dresel, et al., *J. Nucl. Med.* **39(7)**:1138-42 (1998); Tauscher, et al., *Psychopharmacology (Berl).* **141(2)**:175-81 (1999); and Klimke, et al., *Psychiatry Res.* **90(2)**:91-101 (1999).

The methods described herein are particularly useful in diagnosis of CMV in humans, however a broader application of the methods is contemplated by the present invention. For example, suitably labeled compounds can be used in other hosts which serve as models systems for new CMV treatment regimens to follow the spread of CMV in the model systems. Accordingly, the term "host" is meant to include in its broadest sense, any mammal having a CMV infection which expresses US28 on the surface of infected cells.

B. Methods of Treating CMV Infections in a Host

In another aspect, the present invention provides methods of treating CMV infection in a host, by administering to the host an effective amount of a compound which inhibits chemokine binding to US28 on the surface of CMV-infected cells. In this manner, the compound blocks CMV dissemination in the host.

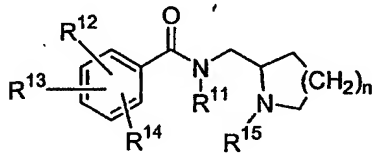
In one group of embodiments, the compounds have the formula:



(I)

or a pharmaceutically acceptable salt thereof; wherein Ar represents a substituted aryl group; R^{11} represents H or (C_1-C_4)alkyl; and N^{Het} is a substituted or unsubstituted 4-, 5-, 6-, or 7-membered nitrogen heterocycle.

Preferred embodiments within this group are those compounds having the formula:



(Ia)

or a pharmaceutically acceptable salt thereof; wherein the subscript n is an integer of from 1 to 3; R^{11} and R^{15} are independently selected from H and substituted or unsubstituted (C_1-C_4)alkyl; R^{12}, R^{13} and R^{14} are each members independently selected from H, halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)haloalkyl, (C_1-C_4)haloalkoxy, nitro, cyano, (C_1-C_4)acyl, amino, (C_1-C_4)alkylamino, and di(C_1-C_4)alkylamino; with the proviso that at least one of R^{12}, R^{13} and R^{14} is other than H.

In certain preferred embodiments within this group, n is 1, R^{11} is H, R^{15} is (C_1-C_4)alkyl; and R^{12}, R^{13} and R^{14} are each other than H. In other preferred embodiments, n is one; R^{11} is H; R^{12}, R^{13} and R^{14} are each independently selected from H, hydroxy, halogen, (C_1-C_4)alkyl and (C_1-C_4)alkoxy; and R^{15} is (C_1-C_4)alkyl.

The methods described herein use the compounds and compositions described herein to treat disease or provide medicinal prophylaxis to individuals who possess a compromised immune system or are expected to suffer immunosuppressed conditions, such as patients prior to undergoing immunosuppressive therapy in connection with organ transplantation or anticancer chemotherapy. These methods generally involve administering to the host an effective amount of the subject compounds or pharmaceutically acceptable compositions.

The compositions and compounds of the invention and the pharmaceutically acceptable salts thereof can be administered in any effective way such as via oral, parenteral or topical routes. Generally, the compounds are administered in dosages ranging from about 2 mg up to about 2,000 mg per day, although variations will necessarily occur depending on the disease target, the patient, and the route of administration. Preferred dosages are administered orally in the range of about 0.05 mg/kg to about 20 mg/kg, more preferably in the range of about 0.05 mg/kg to about 2

mg/kg, most preferably in the range of about 0.05 mg/kg to about 0.2 mg per kg of body weight per day.

Therapeutic and prophylactic methods of this invention comprise the step of treating patients in a pharmaceutically acceptable manner with those compounds or compositions. Such compositions may be in the form of tablets, capsules, caplets, powders, granules, lozenges, suppositories, reconstitutable powders, or liquid preparations, such as oral or sterile parenteral solutions or suspensions. Compounds of the invention may also be administered via an intraocular implant for treating retinitis as a result of CMV infection. In particular, compounds may be embedded in a polymer based implant which will be release into the eye over an extended period of time.

Physicians will determine the dosage of the present therapeutic agents which will be most suitable. Dosages may vary with the mode of administration and the particular compound chosen. In addition, the dosage may vary with the particular patient under treatment. The dosage of the compound used in the treatment will vary, depending on viral load, the weight of the patient, the relative efficacy of the compound and the judgment of the treating physician. Such therapy may extend for several weeks or months, in an intermittent or uninterrupted manner.

C. Compositions useful in the treatment of CMV infection

The present invention also provides compositions useful for preventing CMV dissemination in a host, which comprises a pharmaceutically acceptable carrier or adjuvant and an effective amount of a compound identified using the assays described herein. Preferably, the compound is a compound of formula I, more preferably, formula Ia.

Typically, the compositions contain from about 0.1% to about 99% by weight of active compound, and preferably from about 10% to about 60% by weight depending on which method of administration is employed.

A CMV dissemination-inhibiting amount is that amount of active compound required to slow the progression of viral dissemination or reduce the amount of viral dissemination from that which would otherwise occur without administration of the compound. Or, it is an amount of active compound required to slow the progression or reduce the intensity of symptoms resulting from CMV infection or elimination thereof.

CMV dissemination-inhibiting activity of compounds of the invention can be determined according to the assays described herein. The assays provide an indication of chemokine binding to US28, more typically fractalkine binding to US28. The compounds provided herein inhibit the binding of fractalkine to US28 with activity expressed as IC50 (that amount of compound that reduces fractalkine binding by 50%). The compounds provided herein will typically exhibit an IC50 of approximately 50 μg/mL or less, preferably 25 μg/mL or less, more preferably 10 μg/mL or less, and most preferably less than 1 μg/mL.

For the compositions of the invention, the proportion of each carrier, diluent or adjuvant is determined by the solubility and chemical nature of the compound and the route of administration according to standard pharmaceutical practice. In order to obtain consistency of administration, however, it is preferred that a composition of the invention is in the form of a unit dose. For example, the unit dose presentation forms for oral administration may be tablets and capsules and may contain conventional excipients such as binding agents (e.g., acacia, gelatin, sorbitol, or polyvinylpyrrolidone), fillers (e.g., lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine), tableting lubricants (e.g., magnesium stearate), disintegrants (e.g., starch, polyvinylpyrrolidone, sodium starch glycoallate or microcrystalline cellulose), or pharmaceutically acceptable wetting agents (e.g., sodium lauryl sulfate).

The compounds may be injected parenterally; this being intramuscularly, intravenously, or subcutaneously. For parenteral administration, the compound may be used in the form of sterile solutions containing other solutes, for example, sufficient saline or glucose to make the solution isotonic. The amount of active ingredient administered parenterally will be approximately 0.01 to 250 mg/kg/day, preferably about 1 to 10 mg/kg/day, more preferably about 0.5 to 30 mg/kg/day, and more most preferably about 1-20 mg/kg/day.

The compounds may be administered orally in the form of tablets, capsules, or granules containing suitable excipients such as starch, lactose, white sugar and the like. The compounds may be administered orally in the form of solutions which may contain coloring and/or flavoring agents. The compounds may also be administered sublingually in the form of tracheas or lozenges in which each active ingredient is mixed with sugar or corn syrups, flavoring agents and dyes, and then dehydrated sufficiently to make the mixture suitable for pressing into solid form. The amount of active ingredient administered orally will depend on bioavailability of the specific compound.

The solid oral compositions may be prepared by conventional methods of blending, filling, tableting, or the like. Repeated blending operations may be used to distribute the active agent throughout those compositions employing large quantities of tillers. Such operations are, of course, conventional in the art. The tablets may be coated according to methods well known in normal pharmaceutical practice, in particular with an enteric coating.

Oral liquid preparations may be in the form of emulsions, syrups, or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may or may not contain conventional additives. For example suspending agents, such as sorbitol, syrup, methyl cellulose, gelatin, hydroxyethylcellulose, carboxymethylcellulose, aluminum stearate gel, or hydrogenated edible fats; emulsifying agents, such as sorbitan monooleate or acaci; non-aqueous vehicles (which may include edible oils), such as almond oil, fractionated coconut oil, oily esters selected from the group consisting of glycerin, propylene glycol, ethylene glycol, and ethyl alcohol; preservatives, for instance methyl para-hydroxybenzoate, ethyl para-hydroxybenzoate, n-propyl parahydroxybenzoate, or n-butyl parahydroxybenzoate of sorbic acid; and, if desired, conventional flavoring or coloring agents.

The compounds of the present invention may also be administered in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials are cocoa butter and polyethylene glycols.

For topical use, creams, ointments, jellies, solutions or suspensions, etc., containing the compounds of the present invention are employed. As used herein, topical application is also meant to include the use of mouth washes and gargles.

In another embodiment, the invention provides the subject compounds in the form of a pro-drug, which can be metabolically or chemically converted to the subject compound by the recipient host. A wide variety of pro-drug derivatives are known in the art such as those that rely on hydrolytic cleavage or oxidative activation of the prodrug.

The compositions may be advantageously combined and/or used in combination with other antiviral agents which are either therapeutic or prophylactic agents, and different from the subject compounds. The compositions may also be advantageously combined and/or used in combination with agents that treat or induce

conditions often associated with the viral infections that are sensitive to the present compounds, such as anti-HIV agents or immunosuppressive agents. In many instances, administration in conjunction with the subject compositions enhances the efficacy of such agents. Exemplary antiviral agents include ganciclovir, foscarnet and cidofovir. Exemplary anti-HIV agents include indinavir, ritonavir, AZT, lamivudine and saquinavir. Exemplary immunosuppressive agents include cyclosporin and FK-506. The compositions may also be advantageously used as antiviral prophylactic treatment in combination with immunosuppressive protocols such as bone-marrow destruction (either by radiation or chemotherapy).

To further assist in understanding the present invention, the following non-limiting examples are provided.

EXAMPLES

Example 1

This example describes an assay for evaluating compounds which bind to US28 and inhibit the binding of chemokines. This evaluation can be beneficial in determining suitable dosage levels for either diagnostic methods or methods of treatment.

The US28 expressing cells used in most assays consist of a mouse cell line stably expressing transfected US28 cDNA under the control of a CMV promoter. These cells were cultured in IMDM-5% FBS, and harvested when the concentration was between 0.5-1.0 x 10^6 cells/mL. Some assays were performed with adherent human 293 cells (US28-293 cells) or membranes. The cells were centrifuged and resuspended in assay buffer (20 mM HEPES, 140 mM NaCl, 1mM $CaCl_2$, 5mM $MgCl_2$, and with 0.2% bovine serum albumin) to a concentration of 5.6 x 10^6 cells/mL. Using the Multi-Probe automated system, set up with 8 assay plates at a time, first 0.09 mL of cells was added to the assay plates containing the compounds. The final concentration of the compounds was 5 μg/mL each (1 μg/mL Comgenex). Then 0.09 mL of ^{125}I-fractalkine diluted in assay buffer (final concentration ~2-10fM, with ~30,000 cpm per well) was added, the plates sealed and incubated for approximately 3 hours at 4 degrees C on a shaker

platform. The assay plates were harvested using Packard filter plates, pre-soaked in PEI solution, on the vacuum harvest apparatus. Scintillation fluid (35 μL) was added to all wells, the plates were sealed and counted in a Top Count scintillation counter. Control wells containing either diluent only (for total counts) or excess Fractalkine (1 μg/mL, for non-specific binding) were used to calculate the percent of total inhibition for each set of compounds. Further tests on individual compounds were carried out in the same manner.

Example 2

As secondary assays for compounds that specifically inhibited the binding of radiolabled Fractalkine to US28, cytoplasmic calcium mobilization experiments were done by loading US28-293 cells with INDO-1 dye (45 min. at room temperature), washing with PBS, and resuspending into Ca^{2+} 'flux' buffer (HBSS with 1% fetal bovine serum). For each test, 1 x 10^6 cells were incubated at 37°C in the cuvette of a PTI spectrometer, and the ratio of 410/490 nm emission plotted over time (typically 2-3 minutes), with compounds added at 5 seconds, followed by fractalkine at 60 seconds. A rise in intracellular Ca^{2+} is typically seen when US28-293 cells are challenged with fractalkine, an indication that the US28 receptor bound to the ligand, engaged a G-protein linked cascade which resulted in the mobilization of Ca^{2+} in the cytoplasm of the US28-bearing cells. Compounds which inhibited fractalkine binding were tested in this assay for the effects on Ca^{2+} in this system.

Example 3

This example illustrates the effect of IBZM at inhibiting the binding of fractalkine to US28.

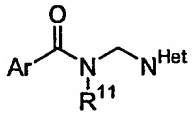
S-(-)-IBZM (from the RBI division of Sigma Chemical Co., St. Louis, Missouri, USA, Catalog No. I-139) was evaluated in the assays described in Examples 1 and 2. A dose response of S(-)-IBZM against fractalkine binding on US28-NSO cells is shown in Figure 1. The IC_{50} value was 0.6 μM. Additionally, when the compound was tested for calcium mobilization in US28-293 cells, IBZM was found to act as a competitive agonist for the US28 receptor, mimicking the action of fractalkine in both binding and signaling (see Figure 2).

In a further study, the binding of IBZM to US28 was shown to be reversible in a competition assay with fractalkine. In this assay, IBZM is pre-incubated with US28 expressing cells (at concentrations of 0-10 μg/mL) and removed by competition with fractalkine (see Figure 3).

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

WHAT IS CLAIMED IS:

1. A method for diagnosis of CMV, said method comprising administering to a subject having CMV, an image-generating amount of a compound having the formula:



or a pharmaceutically acceptable salt thereof; wherein

Ar is a substituted aryl group;

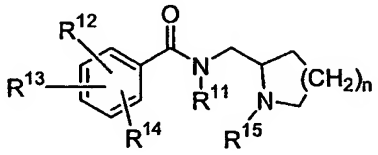
R^{11} is a member selected from the group consisting of H and substituted or unsubstituted $(C_1\text{-}C_4)$alkyl; and

N^{Het} is a substituted or unsubstituted 4-, 5-, 6-, or 7-membered nitrogen heterocycle.

2. A method in accordance with claim 1, wherein Ar is substituted phenyl.

3. A method in accordance with claim 1, wherein Ar is substituted phenyl and N^{Het} is selected from the group consisting of substituted or unsubstituted pyrrolidinyl, substituted or unsubstituted piperazinyl, substituted or unsubstituted piperidinyl, substituted or unsubstituted morpholinyl and substituted or unsubstituted piperidyl.

4. A method in accordance with claim 1, wherein said compound has the formula:



or a pharmaceutically acceptable salt thereof; wherein

the subscript n is an integer of from 1 to 3;

R^{11} and R^{15} are members independently selected from the group consisting of H and substituted or unsubstituted $(C_1\text{-}C_4)$alkyl;

R^{12}, R^{13} and R^{14} are each members independently selected from the group consisting of H, hydroxy, halogen, $(C_1\text{-}C_4)$alkyl, $(C_1\text{-}C_4)$alkoxy, $(C_1$-

C_4)haloalkyl, (C_1-C_4)haloalkoxy, nitro, cyano, (C_1-C_4)acyl, amino, (C_1-C_4)alkylamino and di(C_1-C_4)alkylamino;

with the proviso that at least one of R^{12}, R^{13} and R^{14} is other than H.

5. A method in accordance with claim 1, wherein said compound is labeled with a radioisotope selected from the group consisting of ^{18}F, ^{75}Br, ^{123}I and ^{125}I.

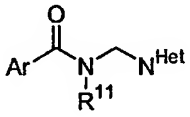
6. A method in accordance with claim 4, wherein n is 1; R^{11} is H; R^{12}, R^{13} and R^{14} are each independently selected from the group consisting of H, hydroxy, halogen, (C_1-C_4)alkyl and (C_1-C_4)alkoxy; and R^{15} is (C_1-C_4)alkyl.

7. A method in accordance with claim 4, wherein said compound is IBZM.

8. A method in accordance with claim 4, wherein said compound is ^{123}I-IBZM.

9. A method for treating CMV in a human, comprising administering an effective amount of a compound which blocks the binding of a chemokine to US28 or a US28 fragment.

10. A method in accordance with claim 9, wherein said compound has the formula:



or a pharmaceutically acceptable salt thereof; wherein

Ar is a substituted aryl group;

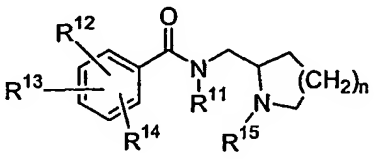
R^{11} is a member selected from the group consisting of H and substituted or unsubstituted (C_1-C_4)alkyl; and

N^{Het} is a substituted or unsubstituted 4-, 5-, 6-, or 7-membered nitrogen heterocycle.

11. A method in accordance with claim 10, wherein Ar is substituted phenyl.

12. A method in accordance with claim 10, wherein Ar is substituted phenyl and N^{Het} is selected from the group consisting of substituted or unsubstituted pyrrolidinyl, substituted or unsubstituted piperazinyl, substituted or unsubstituted piperidinyl, substituted or unsubstituted morpholinyl and substituted or unsubstituted piperidyl.

13. A method in accordance with claim 10, wherein said compound has the formula:



or a pharmaceutically acceptable salt thereof; wherein

the subscript n is an integer of from 1 to 3;

R^{11} and R^{15} are members independently selected from the group consisting of H and substituted or unsubstituted $(C_1\text{-}C_4)$alkyl;

R^{12}, R^{13} and R^{14} are each members independently selected from the group consisting of H, hydroxy, halogen, $(C_1\text{-}C_4)$alkyl, $(C_1\text{-}C_4)$alkoxy, $(C_1\text{-}C_4)$haloalkyl, $(C_1\text{-}C_4)$haloalkoxy, nitro, cyano, $(C_1\text{-}C_4)$acyl, amino, $(C_1\text{-}C_4)$alkylamino and di$(C_1\text{-}C_4)$alkylamino;

with the proviso that at least one of R^{12}, R^{13} and R^{14} is other than H.

14. A method in accordance with claim 13, wherein n is 1, R^{11} is H, R^{15} is $(C_1\text{-}C_4)$alkyl, and R^{12}, R^{13} and R^{14} are all other than H.

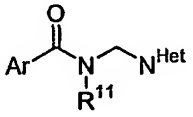
15. A method in accordance with claim 13, wherein n is 1; R^{11} is H; R^{12}, R^{13} and R^{14} are each independently selected from the group consisting of H, hydroxy, halogen, $(C_1\text{-}C_4)$alkyl and $(C_1\text{-}C_4)$alkoxy; and R^{15} is $(C_1\text{-}C_4)$alkyl.

16. A method for reducing cell motility in a CMV-infected cell, said method comprising contacting said CMV-infected cell with a motility-reducing amount of a compound that inhibits chemokine binding to US28 on the surface of said infected cell.

17. A method in accordance with claim 16, wherein said chemokine is a member selected from the group consisting of fractalkine, MIP-1α, MIP-1β, MCP-1 and RANTES.

18. A method in accordance with claim 16, wherein said chemokine is fractalkine.

19. A method in accordance with claim 16, wherein said compound has the formula:



or a pharmaceutically acceptable salt thereof; wherein

Ar is a substituted aryl group;

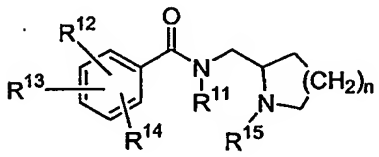
R^{11} is a member selected from the group consisting of H and substituted or unsubstituted $(C_1\text{-}C_4)$alkyl; and

N^{Het} is a substituted or unsubstituted 4-, 5-, 6-, or 7-membered nitrogen heterocycle.

20. A method in accordance with claim 19, wherein Ar is substituted phenyl.

21. A method in accordance with claim 19, wherein Ar is substituted phenyl, and N^{Het} is selected from the group consisting of substituted or unsubstituted pyrrolidinyl, substituted or unsubstituted piperazinyl, substituted or unsubstituted piperidinyl, substituted or unsubstituted morpholinyl and substituted or unsubstituted piperidyl.

22. A method in accordance with claim 16, wherein said compound has the formula:



or a pharmaceutically acceptable salt thereof; wherein

the subscript n is an integer of from 1 to 3;

R^{11} and R^{15} are members independently selected from the group consisting of H and substituted or unsubstituted (C_1-C_4)alkyl;

R^{12}, R^{13} and R^{14} are each members independently selected from the group consisting of H, hydroxy, halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)haloalkyl, (C_1-C_4)haloalkoxy, nitro, cyano, (C_1-C_4)acyl, amino, (C_1-C_4)alkylamino and di(C_1-C_4)alkylamino;

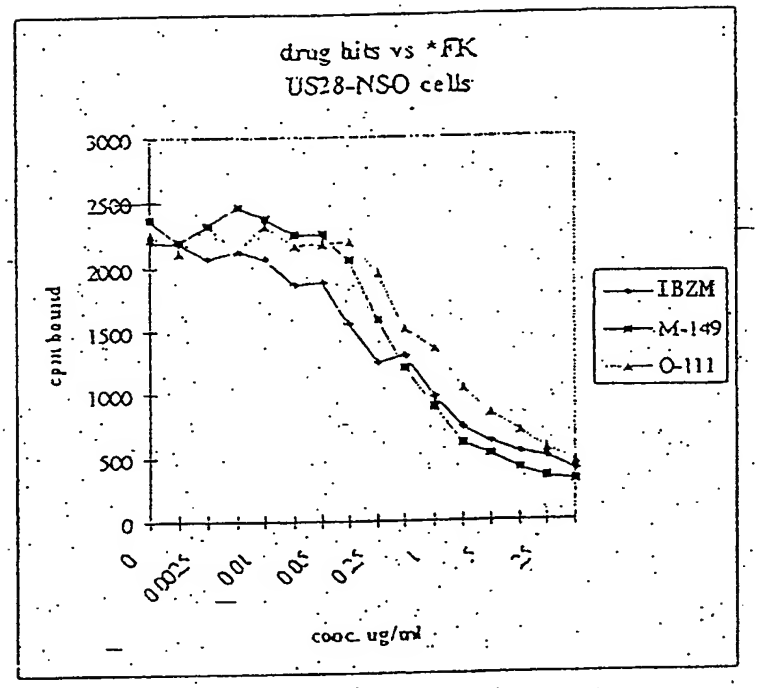
with the proviso that at least one of R^{12}, R^{13} and R^{14} is other than H.

23. A method in accordance with claim 22, wherein n is 1, R^{11} is H, R^{15} is (C_1-C_4)alkyl, and R^{12}, R^{13} and R^{14} are all other than H.

24. A method in accordance with claim 22, wherein n is 1; R^{11} is H; R^{12}, R^{13} and R^{14} are each independently selected from the group consisting of H, hydroxy, halogen, (C_1-C_4)alkyl and (C_1-C_4)alkoxy; and R^{15} is (C_1-C_4)alkyl.

25. A method in accordance with claim 22, wherein said compound is IBZM or a pharmaceutically acceptable salt thereof.

FIGURE 1



The IC_{50} values for each were: M-149, 0.3 μM; IBZM, 0.6 μM; and O-111, 0.7 μM.

FIGURE 2

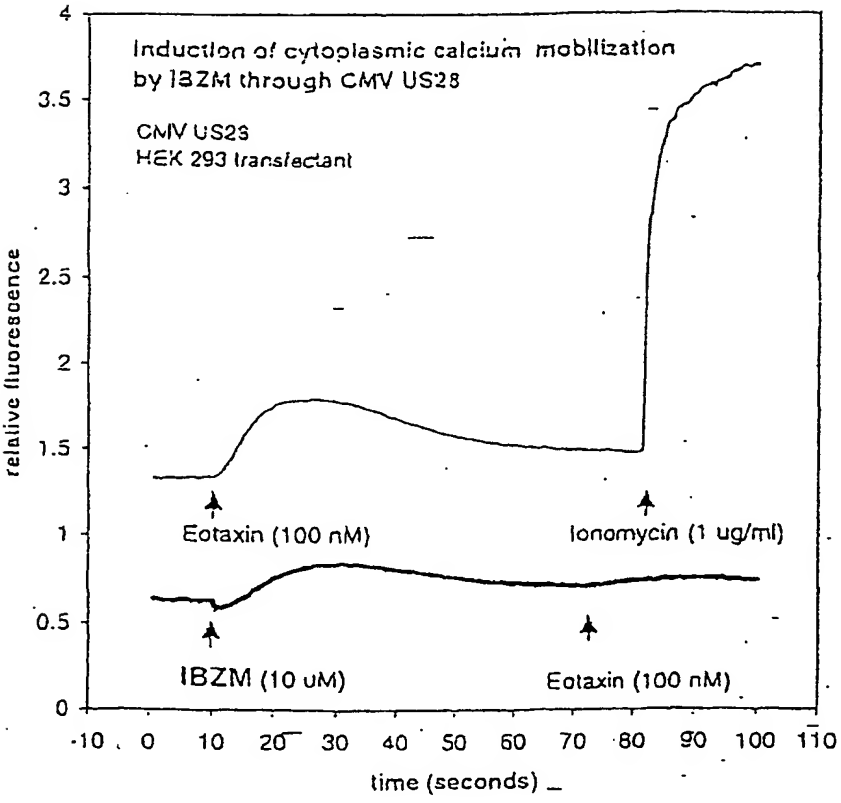
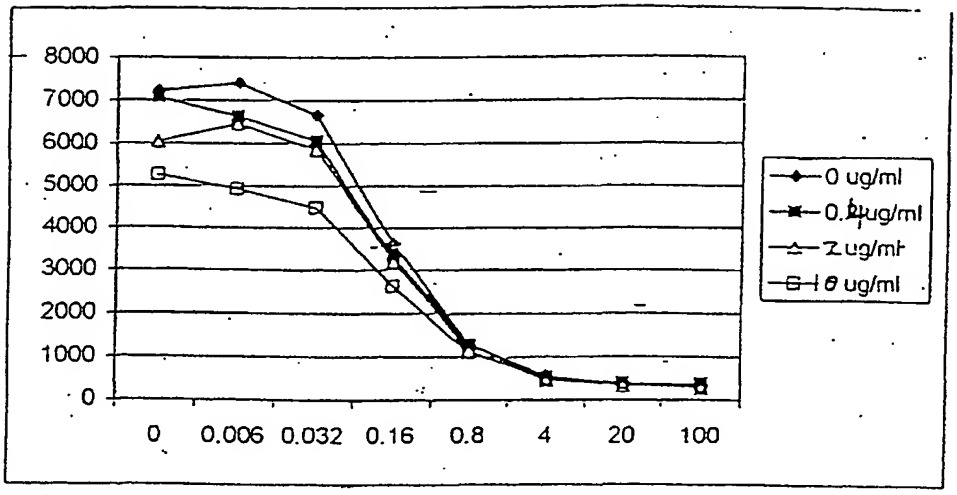

Induction of cytoplasmic calcium mobilization
by IBZM through CMV US28
CMV US28
HEK 293 transfectant
relative fluorescence
4
3.5
3
2.5
2
1.5
1
0.5
0
Eotaxin (100 nM)
Ionomycin (1 ug/ml)
IBZM (10 uM)
Eotaxin (100 nM)
-10
0
10
20
30
40
50
60
70
80
90
100
110
time (seconds)

FIGURE 3


8000
7000
6000
5000
4000
3000
2000
1000
0
0
0.006
0.032
0.16
0.8
4
20
100
0 ug/ml
0.2 ug/ml
2 ug/ml
8 ug/ml

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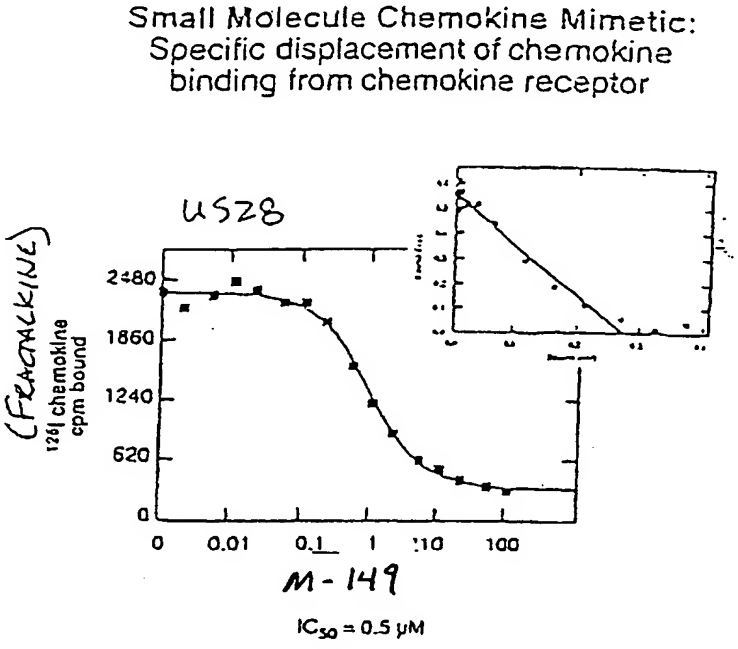
[Continued on next page]

(54) Title: MODULATORS OF US28

(57) Abstract: Assays, compositions and methods of treatment are provided for modulating the binding of chemokines to US28 on the surface of cells.



WO 02/17900 A2

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

MODULATORS OF US28

CROSS-REFERENCES TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Patent Application Serial No. 60/228,974, filed August 30, 2000, and U.S. Provisional Patent Application Serial No. _____, filed August 30, 2001, entitled "Bicyclic Compounds as Inhibitors of Chemokine Binding to US 28" (Attorney Docket No. 019934-001000US), the disclosures of each being incorporated herein by reference. Related subject matter is described in co-owned applications Ser. No. _____, filed August 30, 2001, entitled "Reagents and Methods for the Diagnosis of CMV Dissemination" (Attorney Docket No. 019934-000910US/PCT) which claims the benefit of Ser. No. 60/229,191 filed August 30, 2000; and in Ser. No. _____, filed August 30, 2001, entitled "Inhibition of CMV Infection and Dissemination " (Attorney Docket No. 019934-002510US/PCT) which claims the benefit of Ser. No. 60/229,365, filed August 30, 2000, the disclosures of each being incorporated herein by reference.

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

Not applicable

BACKGROUND OF THE INVENTION

Cytomegalovirus (CMV) is an important human pathogen and a major opportunist which emerges to cause disease in the immuno-compromised such as AIDS patients, neonates, and individuals who have been given immunosuppressive drugs as part of a transplantation regimen. In these individuals, the consequences of CMV in acute or re-emerging infections can be dire, including retinitis, encephalitis, and pneumocystis, among other pathologies. Furthermore, in immuno-competent hosts, CMV establishes a persistent lifelong infection through which it has been linked to a variety of inflammatory conditions including coronary artery occlusion following heart transplant and arthrectomy and restenosis following angioplasty. CMV interacts with leukocytes during acute infection of the host as well as during lifelong latency. As such, leukocytes are important

players in CMV-induced disease and have been implicated in the acute phase of infection as vehicles for dissemination of virus and as sites of residence during lifelong latency.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides an assay for identifying a compound useful for blocking CMV dissemination in a host by determining whether the compound inhibits the binding of a chemokine to US28 or a US28 fragment. Typically, the assay will be run as a competitive binding assay using a labeled chemokine. A variety of chemokines are known to bind to US28 and are useful in this aspect of the invention. Preferably, the chemokine is fractalkine and the assay is a radioligand binding assay.

In another aspect, the present invention provides methods for blocking CMV dissemination in a host by administering to the host an effective amount of a compound which blocks the binding of a chemokine to US28. Preferably, the compound is one which was identified using an assay of the present invention.

In yet another aspect, the present invention provides pharmaceutical compositions for the treatment of CMV comprising compounds identified in the present assays and further described below.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the specific displacement of chemokine (fractalkine) binding to the US28 chemokine receptor.

Figure 2 illustrates the signaling profile and cross desensitization between methiothepin and a chemokine ligand (fractalkine) for US28.

DETAILED DESCRIPTION OF THE INVENTION

Abbreviations and Definitions

The term "alkyl," by itself or as part of another substituent, means, unless otherwise stated, a straight or branched chain, or cyclic hydrocarbon radical, or combination thereof, which may be fully saturated, mono- or polyunsaturated and can include di- and multivalent radicals, having the number of carbon atoms designated (*i.e.*

C_1-C_{10} means one to ten carbons). Examples of saturated hydrocarbon radicals include groups such as methyl, ethyl, n-propyl, isopropyl, n-butyl, t-butyl, isobutyl, sec-butyl, cyclohexyl, (cyclohexyl)methyl, cyclopropylmethyl, homologs and isomers of, for example, n-pentyl, n-hexyl, n-heptyl, n-octyl, and the like. An unsaturated alkyl group is one having one or more double bonds or triple bonds. Examples of unsaturated alkyl groups include vinyl, 2-propenyl, crotyl, 2-isopentenyl, 2-(butadienyl), 2,4-pentadienyl, 3-(1,4-pentadienyl), ethynyl, 1- and 3-propynyl, 3-butynyl, and the higher homologs and isomers. When used alone, the term "alkyl" refers to unsubstituted versions of the radicals indicated above. Substituted forms of "alkyl" are defined in more detail below.

The term "alkylene" by itself or as part of another substituent means a divalent radical derived from an alkane, as exemplified by $-CH_2CH_2CH_2CH_2-$, and further includes those groups described below as "heteroalkylene." Typically, an alkyl (or alkylene) group will have from 1 to 24 carbon atoms, with those groups having 10 or fewer carbon atoms being preferred in the present invention. A "lower alkyl" or "lower alkylene" is a shorter chain alkyl or alkylene group, generally having eight or fewer carbon atoms.

The terms "alkoxy," "alkylamino" and "alkylthio" (or thioalkoxy) are used in their conventional sense, and refer to those alkyl groups attached to the remainder of the molecule via an oxygen atom, an amino group, or a sulfur atom, respectively.

The term "heteroalkyl," by itself or in combination with another term, means, unless otherwise stated, a stable straight or branched chain, or cyclic hydrocarbon radical, or combinations thereof, consisting of the stated number of carbon atoms and from one to three heteroatoms selected from the group consisting of O, N, Si and S, and wherein the nitrogen and sulfur atoms may optionally be oxidized and the nitrogen heteroatom may optionally be quaternized. The heteroatom(s) O, N and S may be placed at any interior position of the heteroalkyl group. The heteroatom Si may be placed at any position of the heteroalkyl group, including the position at which the alkyl group is attached to the remainder of the molecule. Examples include $-CH_2-CH_2-O-CH_3$, $-CH_2-CH_2-NH-CH_3$, $-CH_2-CH_2-N(CH_3)-CH_3$, $-CH_2-S-CH_2-CH_3$, $-CH_2-CH_2-S(O)-CH_3$, $-CH_2-CH_2-S(O)_2-CH_3$, $-CH=CH-O-CH_3$, $-Si(CH_3)_3$, $-CH_2-CH=N-OCH_3$, and $-CH=CH-N(CH_3)-CH_3$. Up to two heteroatoms may be consecutive, such as, for example, $-CH_2-NH-OCH_3$ and $-CH_2-O-Si(CH_3)_3$. Similarly, the term "heteroalkylene" by itself or as part of another substituent means a divalent radical derived from heteroalkyl, as exemplified by $-CH_2-CH_2-S-CH_2CH_2-$ and $-CH_2-S-CH_2-CH_2-NH-CH_2-$. For heteroalkylene groups,

heteroatoms can also occupy either or both of the chain termini (*e.g.*, alkyleneoxy, alkylenedioxy, alkyleneamino, alkylenediamino, and the like). Still further, for alkylene and heteroalkylene linking groups, no orientation of the linking group is implied.

The terms "cycloalkyl" and "heterocycloalkyl", by themselves or in combination with other terms, represent, unless otherwise stated, cyclic versions of "alkyl" and "heteroalkyl", respectively. Additionally, for heterocycloalkyl or heterocyclyl, a heteroatom can occupy the position at which the heterocycle is attached to the remainder of the molecule. Examples of cycloalkyl include cyclopentyl, cyclohexyl, 1-cyclohexenyl, 3-cyclohexenyl, cycloheptyl, and the like. Examples of heterocycloalkyl include 1 -(1,2,5,6-tetrahydropyridyl), 1-piperidinyl, 2-piperidinyl, 3-piperidinyl, 4-morpholinyl, 3-morpholinyl, tetrahydrofuran-2-yl, tetrahydrofuran-3-yl, tetrahydrothien-2-yl, tetrahydrothien-3-yl, 1 -piperazinyl, 2-piperazinyl, and the like.

The terms "halo" or "halogen," by themselves or as part of another substituent, mean, unless otherwise stated, a fluorine, chlorine, bromine, or iodine atom. Additionally, terms such as "haloalkyl," are meant to include monohaloalkyl and polyhaloalkyl. For example, the term "(C_1-C_4)haloalkyl" is mean to include trifluoromethyl, 2,2,2-trifluoroethyl, 4-chlorobutyl, 3-bromopropyl, and the like.

The term "acyl" is used in its conventional sense and refers to an organic radical derived from an organic acid by the removal of the hydroxyl group. Examples of "acyl" groups include acetyl, propionyl, butanoyl, hexanoyl, isobutyryl, octanoyl, and the like.

The term "aryl" means, unless otherwise stated, a polyunsaturated, typically aromatic, hydrocarbon substituent which can be a single ring or multiple rings (up to three rings) which are fused together or linked covalently. The term "heteroaryl" refers to aryl groups (or rings) that contain from zero to four heteroatoms selected from N, O, and S, wherein the nitrogen and sulfur atoms are optionally oxidized, and the nitrogen atom(s) are optionally quaternized. A heteroaryl group can be attached to the remainder of the molecule through a heteroatom. Non-limiting examples of aryl and heteroaryl groups include phenyl, 1-naphthyl, 2-naphthyl, 4-biphenyl, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 3-pyrazolyl, 2-imidazolyl, 4-imidazolyl, pyrazinyl, 2-oxazolyl, 4-oxazolyl, 2-phenyl-4-oxazolyl, 5-oxazolyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl, 5-benzothiazolyl, purinyl, 2-benzimidazolyl, 5-indolyl, 1-isoquinolyl, 5-isoquinolyl, 2-quinoxalinyl, 5-quinoxalinyl, 3-quinolyl, and 6-

quinolyl. Substituents for each of the above noted aryl and heteroaryl ring systems are selected from the group of acceptable substituents described below.

For brevity, the term "aryl" when used in combination with other terms (*e.g.*, aryloxy, arylthioxy, arylalkyl) includes both aryl and heteroaryl rings as defined above. Thus, the term "arylalkyl" is meant to include those radicals in which an aryl group is attached to an alkyl group (*e.g.*, benzyl, phenethyl, pyridylmethyl and the like) including those alkyl groups in which a carbon atom (*e.g.*, a methylene group) has been replaced by, for example, an oxygen atom (*e.g.*, phenoxymethyl, 2-pyridyloxymethyl, 3-(1-naphthyloxy)propyl, and the like).

Each of the above terms (*e.g.*, "alkyl," "heteroalkyl," "aryl" and "heteroaryl") are meant to include both substituted and unsubstituted forms of the indicated radical. Preferred substituents for each type of radical are provided below.

Substituents for the alkyl and heteroalkyl radicals (including those groups often referred to as alkylene, alkenyl, heteroalkylene, heteroalkenyl, alkynyl, cycloalkyl, heterocycloalkyl, cycloalkenyl, and heterocycloalkenyl) can be a variety of groups selected from: -OR', =O, =NR', =N-OR', -NR'R", -SR', -halogen, -SiR'R"R"', -OC(O)R', -C(O)R', -CO_2R', -CONR'R", -OC(O)NR'R", -NR"C(O)R', -NR'-C(O)NR"R"', -NR"$C(O)_2$R', -NH-$C(NH_2)$=NH, -NR'$C(NH_2)$=NH, -NH-$C(NH_2)$=NR', -S(O)R', -$S(O)_2$R', -$S(O)_2$NR'R", -CN and -NO_2 in a number ranging from zero to (2m'+1), where m' is the total number of carbon atoms in such radical. R', R" and R"' each independently refer to hydrogen, unsubstituted (C_1-C_8)alkyl and heteroalkyl, unsubstituted aryl, aryl substituted with 1-3 halogens, unsubstituted alkyl, alkoxy or thioalkoxy groups, or aryl-(C_1-C_4)alkyl groups. When R' and R" are attached to the same nitrogen atom, they can be combined with the nitrogen atom to form a 5-, 6-, or 7-membered ring. For example, -NR'R" is meant to include 1-pyrrolidinyl and 4-morpholinyl. From the above discussion of substituents, one of skill in the art will

system; and where R', R" and R"' are independently selected from hydrogen, $(C_1$-$C_8)$alkyl and heteroalkyl, unsubstituted aryl and heteroaryl, (unsubstituted aryl)-$(C_1$-$C_4)$alkyl, and (unsubstituted aryl)oxy-$(C_1$-$C_4)$alkyl.

Two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula -T-C(O)-$(CH_2)_q$-U-, wherein T and U are independently -NH-, -O-, -CH_2- or a single bond, and q is an integer of from 0 to 2. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula -A-$(CH_2)_r$-B-, wherein A and B are independently -CH_2-, -O-, -NH-, -S-, -S(O)-, -$S(O)_2$-, -$S(O)_2$NR'- or a single bond, and r is an integer of from 1 to 3. One of the single bonds of the new ring so formed may optionally be replaced with a double bond. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula -$(CH_2)_s$-X-$(CH_2)_t$-, where s and t are independently integers of from 0 to 3, and X is -O-, -NR'-, -S-, -S(O)-, -$S(O)_2$-, or -$S(O)_2$NR'-. The substituent R' in -NR'- and -$S(O)_2$NR'- is selected from hydrogen or unsubstituted $(C_1$-$C_6)$alkyl.

As used herein, the term "heteroatom" is meant to include oxygen (O), nitrogen (N), sulfur (S) and silicon (Si).

The term "pharmaceutically acceptable salts" is meant to include salts of the active compounds which are prepared with relatively nontoxic acids or bases, depending on the particular substituents found on the compounds described herein. When compounds of the present invention contain relatively acidic functionalities, base addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired base, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable base addition salts include sodium, potassium, calcium, ammonium, organic amino, or magnesium salt, or a similar salt. When compounds of the present invention contain relatively basic functionalities, acid addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired acid, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable acid addition salts include those derived from inorganic acids like hydrochloric, hydrobromic, nitric, carbonic, monohydrogencarbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulfuric, monohydrogensulfuric, hydriodic, or phosphorous acids and the like, as well as the salts derived from relatively nontoxic organic acids like acetic, propionic, isobutyric, maleic, malonic, benzoic,

succinic, suberic, fumaric, mandelic, phthalic, benzenesulfonic, p-tolylsulfonic, citric, tartaric, methanesulfonic, and the like. Also included are salts of amino acids such as arginate and the like, and salts of organic acids like glucuronic or galactunoric acids and the like (see, for example, Berge, S.M., et al, "Pharmaceutical Salts", *Journal of Pharmaceutical Science*, 1977, *66*, 1-19). Certain specific compounds of the present invention contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts.

The neutral forms of the compounds may be regenerated by contacting the salt with a base or acid and isolating the parent compound in the conventional manner. The parent form of the compound differs from the various salt forms in certain physical properties, such as solubility in polar solvents, but otherwise the salts are equivalent to the parent form of the compound for the purposes of the present invention.

In addition to salt forms, the present invention provides compounds which are in a prodrug form. Prodrugs of the compounds described herein are those compounds that readily undergo chemical changes under physiological conditions to provide the compounds of the present invention. Additionally, prodrugs can be converted to the compounds of the present invention by chemical or biochemical methods in an *ex vivo* environment. For example, prodrugs can be slowly converted to the compounds of the present invention when placed in a transdermal patch reservoir with a suitable enzyme or chemical reagent.

Certain compounds of the present invention can exist in unsolvated forms as well as solvated forms, including hydrated forms. In general, the solvated forms are equivalent to unsolvated forms and are intended to be encompassed within the scope of the present invention. Certain compounds of the present invention may exist in multiple crystalline or amorphous forms. In general, all physical forms are equivalent for the uses contemplated by the present invention and are intended to be within the scope of the present invention.

Certain compounds of the present invention possess asymmetric carbon atoms (optical centers) or double bonds; the racemates, diastereomers, geometric isomers and individual isomers are all intended to be encompassed within the scope of the present invention.

The compounds of the present invention may also contain unnatural proportions of atomic isotopes at one or more of the atoms that constitute such compounds. For example, the compounds may be radiolabeled with radioactive isotopes,

such as for example tritium (^{3}H), iodine-125 (^{125}I) or carbon-14 (^{14}C). All isotopic variations of the compounds of the present invention, whether radioactive or not, are intended to be encompassed within the scope of the present invention.

General

CMV harbors in its genome an open reading frame (ORF), designated US28, which encodes a protein that acts as a functional receptor for certain human and viral chemokines. Upon infection of a cell by CMV, US28 is expressed on the surface of the infected cell and becomes capable of responding to chemokines in the environment. Because the virus on its own is inherently non-motile, and because chemokines and their receptors encoded by human cells are known to regulate the migration of leukocytes and other cells through the body, CMV US28 is now thought to be encoded by the virus to facilitate the dissemination of CMV through the body during and after infection. Therefore, agents which block the binding of chemokines to US28 are expected to be useful in inhibiting viral dissemination during acute or re-emerging CMV infection.

CMV US28 has been shown to bind a variety of human, murine , and virus-encoded CC chemokines in a variety of assay formats. In addition, the CX3C chemokine, Fractalkine, binds with a very high affinity (K_I~50 pM) to US28. Fractalkine is expressed on certain endothelial cell surfaces and on populations of dendritic cells (DC), and may thus define a portal through which CMV infected cells go from the circulation to the tissue space, as well as find residence in the DC.

Since the US28 receptor is expressed on cytomegalovirus infected cells, and also in view of its ability to bind multiple chemokines, a small molecule inhibitor for this receptor would have significant use as an anti-CMV agent.

Accordingly, the present invention provides a novel mechanism for control of cytomegalovirus induced disease. By inhibiting dissemination of virus from sites of primary or recurrent infection, the compounds described herein can limit the viral spread to secondary organs and so limit viral replication. Unlike current herpes antiviral agents, the compounds described herein do not act at the stage of viral DNA replication and so are less prone to problems with toxicity and the development of viral resistance. Other GPCR targeted therapeutics have demonstrated high efficacy and been well tolerated for a number of indications.

Description of the Embodiments

A. Assays for identifying compounds which block viral dissemination

In one aspect, the present invention provides assays for identifying a compound capable of blocking CMV dissemination in a host, by determining whether the compound inhibits the binding of a chemokine to US28 or a US28 fragment.

The assays provided herein are typically cell-based assays in which a cell which stably expresses US28 is treated with a candidate compound and a chemokine in a competitive binding format. A variety of other assay formats are also useful in the present invention. For example, substrate-bound or support-bound chemokines (or ligands) can be contacted with a labeled cell or liposome having an associated US28 or US28 fragment

A variety of cell lines can be used in this aspect of the invention. In one group of embodiments, the cell line is a mouse cell line (e.g., NSO cells from R&D Systems, Minneapolis, Minnesota, USA). In other embodiments, the cell line is a human cell line (e.g., primary human lung and foreskin fibroblasts from Clonetics, San Diego California, USA, or human diploid lung fibroblasts (MRC-5 and WI-38), or HUVECs). Additionally, human embryonic kidney 293 cells ("HEK293" from American Tissue Culture Collection) can also be used. In still other embodiments, the cell line is a primary rhesus monkey dermal fibroblast (from University of California at Davis Primate Center). In each instance, the cell lines described can be infected with whole virus (CMV) or transfected with US28 cDNA, typically under the control of a CMV promoter, using conventional methods. Alternatively, cell-free systems can also be employed wherein a fragment of US28 (e.g., NH_2-terminal peptide, extracellular loops and the like) can be used alone (or in combinations of US28 fragments) to assay binding levels of a chemokine in the presence of a candidate agent. In still other embodiments, expressed or synthesized receptor proteins of US28 can be embedded in artificial membrane systems to assay for chemokine binding in the presence of a candidate agent (see for example, systems described in Kitaguchi, et al., *Biochem. Biophys. Res. Commun.* **261(3)**:784-789 (1999) and Myung, et al., *Anal. Biochem.* **270(2)**:303-313 (1999)).

For assays using cells, the cells are cultured in a suitable buffer (e.g., IMDM-5% FBS, DMEM 1885-10% FCS, HUVEC complete medium, and the like) then

centrifuged and resuspended in assay buffer (e.g., HEPES with NaCl, $CaCl_2$, $MgCl_2$, and BSA) to a concentration of from about 5×10^5 to about 5×10^7, preferably from about 2 to about 8×10^6. Aliquots of the cells are then contacted with the candidate compounds and labeled chemokine.

A variety of chemokines can be used in this aspect of the invention, including, for example, fractalkine, RANTES, MCP-3, MIP-1α and MCP-1. A number of the chemokines are commercially available from sources such as R&D Systems or Peprotech, Inc., New Jersey, USA. Preferably, the labeled chemokine is labeled fractalkine. Additionally, a variety of labels can also be used with the chemokines described above. Typically, the label will be a fluorescence label, a phosphorescence label, a radiolabel, a colorimetric label, or the like. In preferred embodiments the labeled chemokine is a radiolabeled fractalkine, more preferably, ^{125}I-fractalkine.

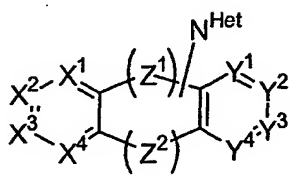
After contacting the cells with one or more candidate compounds in the presence of labeled chemokine, the assay mixture is typically incubated for a period of time of from about 1 to about 6 hours at a temperature of from about 1 to about 10°C. Preferably the mixture is incubated for a period of from about 2 to about 4 hours at a temperature of about 4°C. One of skill in the art will understand that a variety of assay conditions can be employed, depending on the cell line used, the concentrations of the compounds and chemokine and the concentration of the cells themselves.

Following incubation the assay wells can be harvested under vacuum using filter plates, pre-soaked with PEI solution (for those embodiments carried out on 96-, 384-, 1536-well or larger plates). Scintillation fluid (for radiolabel assays) is added, the plates are sealed and the wells are counted. Alternatively, other quantitative methods are employed when, for example, fluorescent labels are used.

B. Compounds which block CMV dissemination

Using the assays described herein, compounds have now been identified which block CMV dissemination.

In one group of embodiments, the compounds have the formula:



(I)

wherein X^1, X^2, X^3 and X^4 are each independently N or C-R^1, wherein R^1 is H, halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkylthio, (C_1-C_4)haloalkyl, (C_1-C_4)haloalkoxy, nitro, cyano, (C_1-C_4)acyl, amino, (C_1-C_4)alkylamino, or di(C_1-C_4)alkylamino. Similarly, Y^1, Y^2, Y^3 and Y^4 are each independently N or C-R^2, wherein R^2 is H, halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkylthio, (C_1-C_4)haloalkyl, (C_1-C_4)haloalkoxy, nitro, cyano, (C_1-C_4)acyl, amino, (C_1-C_4)alkylamino, or di(C_1-C_4)alkylamino.

The symbol Z^1 represents a substituted or unsubstituted (C_1-C_3)alkylene. The symbol Z^2 represents a divalent moiety selected from –O-, -S- and -N(R)- wherein R is H, halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)haloalkyl, (C_1-C_4)haloalkoxy, nitro, cyano, (C_1-C_4)acyl, amino, (C_1-C_4)alkylamino, or di(C_1-C_4)alkylamino.

The symbol N^{Het} represents a substituted or unsubstituted 4-, 5-, 6-, or 7-membered nitrogen heterocycle.

In preferred embodiments, at least two of X^1, X^2, X^3 and X^4 are CH, more preferably three of X^1, X^2, X^3 and X^4 are CH and the fourth is C-R^1, wherein R^1 is halogen, (C_1-C_4)alkylthio, (C_1-C_4)haloalkyl, (C_1-C_4)haloalkoxy, nitro, cyano, or (C_1-C_4)acyl. Also preferred are those embodiments in which Y^1, Y^2, Y^3 and Y^4 are each independently C-R^2, wherein R^2 is H, halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkylthio, (C_1-C_4)haloalkyl, (C_1-C_4)haloalkoxy, nitro, cyano, (C_1-C_4)acyl, amino, (C_1-C_4)alkylamino, di(C_1-C_4)alkylamino. More preferably, each of Y^1, Y^2, Y^3 and Y^4 are independently C-R^2, wherein R^2 is H, halogen, (C_1-C_4)alkylthio, or (C_1-C_4)haloalkyl.

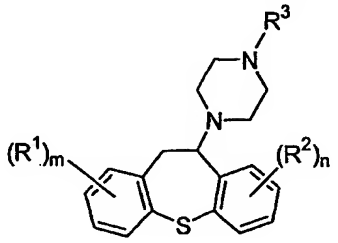
In other preferred embodiments, Z^1 represents an ethylene or propylene group, more preferably an ethylene group in which N^{Het} is attached at the position adjacent to the ring defined by Y^1, Y^2, Y^3 and Y^4.

Also preferred are those embodiments in which Z^2 is –O- or -S-, more preferably –S-.

Preferred groups for N^{Het} are the substituted or unsubstituted 5- or 6-membered nitrogen heterocycles. Particularly preferred heterocycles include piperidine, piperazine, pyrrolidine, oxazoline, imidazoline, pyrazine and morpholine.

More preferably, N^{Het} is a substituted or unsubstituted 6-membered nitrogen heterocycle. In the most preferred embodiments, N^{Het} is a substituted or unsubstituted piperazine which is attached to Z^1 through a nitrogen atom of the piperazine ring. Preferred substituents for the piperazine ring are (C_1-C_4)alkyl, (C_1-C_4)haloalkyl, or (C_1-C_4)acyl. Further preferred substitutents are (C_1-C_4)alkyl, with methyl, ethyl and propyl substituents being the most preferred.

In the most preferred embodiments, the compounds are substituted 10-piperazino-10,11-dihydrodibenzo(b,f)thiepins having the formula:



(Ia)

wherein the subscripts m and n are independently integers of from 0 to 3, preferably 0 to 2, more preferably 0 or 1; and R^1 and R^2 are substituents independently selected from the group of halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkylthio, (C_1-C_4)haloalkyl, (C_1-C_4)haloalkoxy, nitro, cyano, (C_1-C_4)acyl, amino, (C_1-C_4)alkylamino, and di(C_1-C_4)alkylamino. The symbol R^3 represents (C_1-C_4)alkyl, (C_1-C_4)haloalkyl, or (C_1-C_4)acyl.

In particularly preferred embodiments, m is 0 and n is 1. More preferably, m is 0, n is 1 and R^2 is selected from the group of halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkylthio and (C_1-C_4)haloalkyl. Still further preferred are those embodiments in which m is 0, n is 1 and R^2 is selected from the group of halogen and (C_1-C_4)alkylthio. Most preferably, the R^2 substituent is at the 8-position of the dihydrodibenzo(b,f)thiepin ring system.

Particularly preferred compounds for use in the present invention are methiothepin (free base or salt, CAS No. 20229-30-5) and octoclothepin (free base or salt, CAS No. 4789-68-8, for the maleate salt).

Other suitable compounds for use in the present invention (compositions and methods) are described in U.S. Patent No. 3,379,729 "Piperazinyldibenzothiepins" April 23, 1968. See also U.S. Patent No. 4,444,778. Still other related and useful dihydrodibenzo(b,f)thiepins are described in Jilek, et al., *Collect. Czech. Chem. Commun.* **33(6)**:1831-1845 (1968).

C. Compositions useful in the treatment of CMV infection

The present invention also provides compositions useful for preventing CMV dissemination in a host, which comprises a pharmaceutically acceptable carrier or adjuvant and an effective amount of a compound identified using the assays described herein. Preferably, the compound is a compound of formula I, more preferably a compound of formula Ia. Other preferred compounds are those described in Provisional Application Ser. No. _____, filed August 30, 2001 entitled "Bicyclic Compounds as Inhibitors of Chemokine Binding to US 28", incorporated herein by reference. Particularly preferred compounds are those exemplified in the tables of the noted application.

Typically, the compositions contain from about 0.1% to about 99% by weight of active compound, and preferably from about 10% to about 60% by weight depending on which method of administration is employed.

A CMV dissemination-inhibiting amount is that amount of active compound required to slow the progression of viral dissemination or reduce the amount of viral dissemination from that which would otherwise occur without administration of the compound. Or, it is an amount of active compound required to slow the progression or reduce the intensity of symptoms resulting from CMV infection or elimination thereof.

CMV dissemination-inhibiting activity of compounds of the invention can be determined according to the assays described herein. The assays provide an indication of chemokine binding to US28, more typically fractalkine binding to US28. The compounds provided herein inhibit the binding of fractalkine to US28 with activity expressed as IC50 (that amount of compound that reduces fractalkine binding by 50%). The compounds provided herein will typically exhibit an IC50 of approximately 50 μg/mL or less, preferably 25 μg/mL or less, more preferably 10 μg/mL or less, and most preferably less than 1 μg/mL.

For the compositions of the invention, the proportion of each carrier, diluent or adjuvant is determined by the solubility and chemical nature of the compound and the route of administration according to standard pharmaceutical practice. In order to obtain consistency of administration, however, it is preferred that a composition of the invention is in the form of a unit dose. For example, the unit dose presentation forms for oral administration may be tablets and capsules and may contain conventional excipients such as binding agents (e.g., acacia, gelatin, sorbitol, or polyvinylpyrrolidone), fillers

(e.g., lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine), tableting lubricants (e.g., magnesium stearate), disintegrants (e.g., starch, polyvinylpyrrolidone, sodium starch glycoallate or microcrystalline cellulose), or pharmaceutically acceptable wetting agents (e.g., sodium lauryl sulfate).

The compounds may be injected parenterally; this being intramuscularly, intravenously, or subcutaneously. For parenteral administration, the compound may be used in the form of sterile solutions containing other solutes, for example, sufficient saline or glucose to make the solution isotonic. The amount of active ingredient administered parenterally will be approximately 0.01 to 250 mg/kg/day, preferably about 1 to 10 mg/kg/day, more preferably about 0.5 to 30 mg/kg/day, and more most preferably about 1-20 mg/kg/day.

The compounds may be administered orally in the form of tablets, capsules, or granules containing suitable excipients such as starch, lactose, white sugar and the like. The compounds may be administered orally in the form of solutions which may contain coloring and/or flavoring agents. The compounds may also be administered sublingually in the form of tracheas or lozenges in which each active ingredient is mixed with sugar or corn syrups, flavoring agents and dyes, and then dehydrated sufficiently to make the mixture suitable for pressing into solid form. The amount of active ingredient administered orally will depend on bioavailability of the specific compound.

The solid oral compositions may be prepared by conventional methods of blending, filling, tableting, or the like. Repeated blending operations may be used to distribute the active agent throughout those compositions employing large quantities of tillers. Such operations are, of course, conventional in the art. The tablets may be coated according to methods well known in normal pharmaceutical practice, in particular with an enteric coating.

Oral liquid preparations may be in the form of emulsions, syrups, or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may or may not contain conventional additives. For example suspending agents, such as sorbitol, syrup, methyl cellulose, gelatin, hydroxyethylcellulose, carboxymethylcellulose, aluminum stearate gel, or hydrogenated edible fats; emulsifying agents, such as sorbitan monooleate or acaci; non-aqueous vehicles (which may include edible oils), such as almond oil, fractionated coconut oil, oily esters selected from the group consisting of glycerin, propylene glycol, ethylene glycol, and ethyl alcohol; preservatives, for instance methyl para-

hydroxybenzoate, ethyl para-hydroxybenzoate, n-propyl parahydroxybenzoate, or n-butyl parahydroxybenzoate of sorbic acid; and, if desired, conventional flavoring or coloring agents.

The compounds of the present invention may also be administered in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials are cocoa butter and polyethylene glycols.

For topical use, creams, ointments, jellies, solutions or suspensions, etc., containing the compounds of the present invention are employed. As used herein, topical application is also meant to include the use of mouth washes and gargles.

In another embodiment, the invention provides the subject compounds in the form of a pro-drug, which can be metabolically or chemically converted to the subject compound by the recipient host. A wide variety of pro-drug derivatives are known in the art such as those that rely on hydrolytic cleavage or oxidative activation of the prodrug.

The compositions may be advantageously combined and/or used in combination with other antiviral agents which are either therapeutic or prophylactic agents, and different from the subject compounds. The compositions may also be advantageously combined and/or used in combination with agents that treat or induce conditions often associated with the viral infections that are sensitive to the present compounds, such as anti-HIV agents or immunosuppressive agents. In many instances, administration in conjunction with the subject compositions enhances the efficacy of such agents. Exemplary antiviral agents include ganciclovir, foscarnet and cidofovir. Exemplary anti-HIV agents include indinavir, ritonavir, AZT, lamivudine and saquinavir. Exemplary immunosuppressive agents include cyclosporin and FK-506. The compositions may also be advantageously used as antiviral prophylactic treatment in combination with immunosuppressive protocols such as bone-marrow destruction (either by radiation or chemotherapy).

D. Methods of treating CMV infection

In yet another aspect, the present invention provides novel methods for the use of the foregoing compounds and compositions. In particular, the invention provides novel methods for treating or preventing viral dissemination from CMV infection. The

methods typically involve administering to a patient an effective formulation of one or more of the subject compositions.

The invention provides methods of using the subject compounds and compositions to treat disease or provide medicinal prophylaxis to individuals who possess a compromised immune system or are expected to suffer immunosuppressed conditions, such as patients prior to undergoing immunosuppressive therapy in connection with organ transplantation or anticancer chemotherapy. These methods generally involve administering to the host an effective amount of the subject compounds or pharmaceutically acceptable compositions.

The compositions and compounds of the invention and the pharmaceutically acceptable salts thereof can be administered in any effective way such as via oral, parenteral or topical routes. Generally, the compounds are administered in dosages ranging from about 2 mg up to about 2,000 mg per day, although variations will necessarily occur depending on the disease target, the patient, and the route of administration. Preferred dosages are administered orally in the range of about 0.05 mg/kg to about 20 mg/kg, more preferably in the range of about 0.05 mg/kg to about 2 mg/kg, most preferably in the range of about 0.05 mg/kg to about 0.2 mg per kg of body weight per day.

Therapeutic and prophylactic methods of this invention comprise the step of treating patients in a pharmaceutically acceptable manner with those compounds or compositions. Such compositions may be in the form of tablets, capsules, caplets, powders, granules, lozenges, suppositories, reconstitutable powders, or liquid preparations, such as oral or sterile parenteral solutions or suspensions. Compounds of the invention may also be administered via an intraocular implant for treating retinitis as a result of CMV infection. In particular, compounds may be embedded in a polymer based implant which will be release into the eye over an extended period of time.

Physicians will determine the dosage of the present therapeutic agents which will be most suitable. Dosages may vary with the mode of administration and the particular compound chosen. In addition, the dosage may vary with the particular patient under treatment. The dosage of the compound used in the treatment will vary, depending on viral load, the weight of the patient, the relative efficacy of the compound and the judgment of the treating physician. Such therapy may extend for several weeks or months, in an intermittent or uninterrupted manner.

To further assist in understanding the present invention, the following non-limiting examples are provided.

EXAMPLES

Example 1

The US28 expressing cells used in most assays consist of a mouse cell line (NSO cells from ATCC) stably expressing transfected US28 cDNA under the control of a CMV promoter (from R & D Systems). These cells were cultured in IMDM-5% FBS, and harvested when the concentration was between 0.5-1.0 x 10^6 cells/mL. Some assays were performed with adherent human 293 cells (US28-293 cells) or membranes. The cells were centrifuged and resuspended in assay buffer (20 mM HEPES, 140 mM NaCl, 1mM $CaCl_2$, 5mM $MgCl_2$, and with 0.2% bovine serum albumin) to a concentration of 5.6 x 10^6 cells/mL. Using the Multi-Probe automated system, set up with 8 assay plates at a time, first 0.09 mL of cells was added to the assay plates containing the compounds. The final concentration of the compounds was 5 μg/mL each. Then 0.09 mL of ^{125}I-fractalkine diluted in assay buffer (final concentration ~2-10fM, with ~30,000 cpm per well) was added, the plates sealed and incubated for approximately 3 hours at 4 degrees C on a shaker platform. The assay plates were harvested using Packard filter plates, pre-soaked in PEI solution, on the vacuum harvest apparatus. Scintillation fluid (35 μL) was added to all wells, the plates were sealed and counted in a Top Count scintillation counter. Control wells containing either diluent only (for total counts) or excess Fractalkine (1 μg/mL, for non-specific binding) were used to calculate the percent of total inhibition for each set of compounds. Further tests on individual compounds were carried out in the same manner.

Example 2

As secondary assays for compounds that specifically inhibited the binding of radiolabled Fractalkine to US28, cytoplasmic calcium mobilization experiments were done by loading US28-293 cells with INDO-1 dye (45 min. at room temperature),

washing with PBS, and resuspending into Ca2+ 'flux' buffer (HBSS with 1% fetal bovine serum). For each test, 1 x 10^6 cells were incubated at 37°C in the cuvette of a PTI spectrometer, and the ratio of 410/490 nm emission plotted over time (typically 2-3 minutes), with compounds added at 5 seconds, followed by fractalkine at 60 seconds. A rise in intracellular Ca2+ is typically seen when US28-293 cells are challenged with fractalkine, an indication that the US28 receptor bound to the ligand, engaged a G-protein linked cascade which resulted in the mobilization of Ca2+ in the cytoplasm of the US28-bearing cells. Compounds which inhibited fractalkine binding were tested in this assay for the effects on Ca2+ in this system.

Example 3

This example illustrates the effects of octoclothepin and methiothepin at inhibiting the binding of fractalkine to US28.

Methiothepin mesylate (from the RBI division of Sigma Chemical Co., St. Louis, Missouri, USA, Catalog No. M-149) and octoclothepin maleate (from RBI, Catalog No. O-111) were evaluated in the assays described in Examples 1 and 2. A dose response of methiothepin mesylate and octoclothepin maleate against fractalkine on US28-NSO cells is shown in Figure 1. The IC_{50} values were 0.3 μM for methiothepin mesylate and 0.7 μM for octoclothepin maleate. Additionally, when the compounds were tested for calcium mobilization in US28-293 cells, both compounds were found to act as competitive agonists for the US28 receptor, mimicking the action of fractalkine in both binding and signaling (see Figure 2).

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

WHAT IS CLAIMED IS:

1. An assay for identifying a compound useful for blocking CMV dissemination is a host, comprising the step of determining whether said compound inhibits the binding of a chemokine to US28 or a US28 fragment.

2. An assay in accordance with claim 1, wherein said chemokine is selected from the group consisting of fractalkine, MIP-1α, MIP-1β, MCP-1 and RANTES.

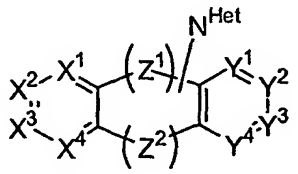
3. An assay in accordance with claim 1, wherein said chemokine is fractalkine.

4. An assay in accordance with claim 1, wherein said step of determining comprises specifically binding labeled fractalkine to the ligand binding domain of US28.

5. A method for preventing dissemination of CMV in a human, comprising administering an effective amount of a compound which blocks the binding of a chemokine to US28 or a US28 fragment.

6. A method in accordance with claim 5, wherein said compound was identified by the assay of claim 1.

7. A method in accordance with claim 5, wherein said compound has the formula:



wherein

X^1, X^2, X^3 and X^4 are each independently members selected from the group consisting of N and C-R^1, wherein R^1 is a member selected from the group consisting of H, halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)haloalkyl, (C_1-C_4)haloalkoxy, nitro, cyano, (C_1-C_4)acyl, amino, (C_1-C_4)alkylamino, and di(C_1-C_4)alkylamino;

Y^1, Y^2, Y^3 and Y^4 are each independently members selected from the group consisting of N and C-R^2, wherein R^2 is a member selected from the group consisting of H, halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)haloalkyl, (C_1-C_4)haloalkoxy, nitro, cyano, (C_1-C_4)acyl, amino, (C_1-C_4)alkylamino, and di(C_1-C_4)alkylamino;

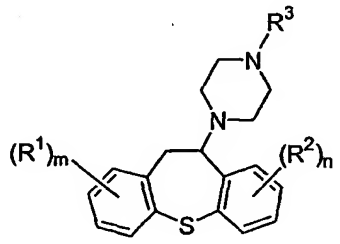
Z^1 is a divalent moiety selected from the group consisting of (C_1-C_3)alkylene;

Z^2 is a divalent moiety selected from the group consisting of –O-, -S- and -N(R^3)- wherein R^3 is a member selected from the group consisting of H, halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)haloalkyl, (C_1-C_4)haloalkoxy, nitro, cyano, (C_1-C_4)acyl, amino, (C_1-C_4)alkylamino, and di(C_1-C_4)alkylamino; and

N^{Het} is a substituted or unsubstituted 4-, 5-, 6-, or 7-membered nitrogen heterocycle.

8. A method in accordance with claim 7, wherein X^1, X^3, X^4, Y^1, Y^2, Y^3 and Y^4 are all CH; Z^2 is –S-, and N^{Het} is a substituted 6-membered nitrogen heterocycle.

9. A method in accordance with claim 5, wherein said compound has the formula:



wherein

the subscripts m and n are independently integers of from 0 to 3;

R^1 and R^2 are substituents independently selected from the group consisting of halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkylthio, (C_1-C_4)haloalkyl, (C_1-C_4)haloalkoxy, nitro, cyano, (C_1-C_4)acyl, amino, (C_1-C_4)alkylamino, and di(C_1-C_4)alkylamino; and

R^3 is a substituent selected from the group consisting of (C_1-C_4)alkyl, (C_1-C_4)haloalkyl and (C_1-C_4)acyl.

10. A method in accordance with claim 9, wherein m is 0 and n is 1.

11. A method in accordance with claim 9, wherein m is 0, n is 1 and R^2 is selected from the group consisting of halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkylthio and (C_1-C_4)haloalkyl.

12. A method in accordance with claim 9, wherein m is 0, n is 1 and R^2 is selected from the group consisting of halogen and (C_1-C_4)alkylthio.

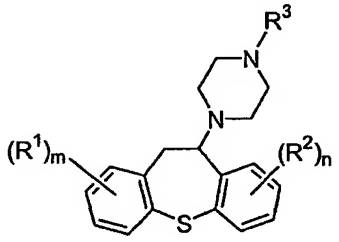
13. A method in accordance with claim 5, wherein said compound is selected from the group consisting of methiothepin, octoclothepin and pharmaceutically acceptable salts thereof.

14. A method for reducing cell motility in a CMV-infected cell, said method comprising contacting said CMV-infected cell with a motility-reducing amount of a compound that inhibits chemokine binding to US28 on the surface of said infected cell.

15. A method in accordance with claim 14, wherein said chemokine is a member selected from the group consisting of fractalkine, MIP-1α, MIP-1β, MCP-1 and RANTES.

16. A method in accordance with claim 14, wherein said chemokine is fractalkine.

17. A method in accordance with claim 14, wherein said compound has the formula:



wherein

the subscripts m and n are independently integers of from 0 to 3;

R^1 and R^2 are substituents independently selected from the group consisting of halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkylthio, (C_1-C_4)haloalkyl, (C_1-C_4)haloalkoxy, nitro, cyano, (C_1-C_4)acyl, amino, (C_1-C_4)alkylamino, and di(C_1-C_4)alkylamino; and

R^3 is a substituent selected from the group consisting of (C_1-C_4)alkyl, (C_1-C_4)haloalkyl and (C_1-C_4)acyl.

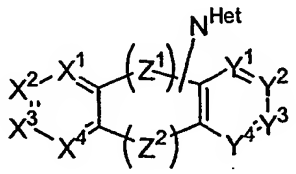
18. A method in accordance with claim 17, wherein m is 0 and n is 1.

19. A method in accordance with claim 17, wherein m is 0, n is 1 and R^2 is selected from the group consisting of halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkylthio and (C_1-C_4)haloalkyl.

20. A method in accordance with claim 17, wherein m is 0, n is 1 and R^2 is selected from the group consisting of halogen and (C_1-C_4)alkylthio.

21. A method in accordance with claim 14, wherein said compound is selected from the group consisting of methiothepin, octoclothepin and pharmaceutically acceptable salts thereof.

22. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a compound of the formula:



wherein

X^1, X^2, X^3 and X^4 are each independently members selected from the group consisting of N and C-R^1, wherein R^1 is a member selected from the group consisting of H, halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)haloalkyl, (C_1-C_4)haloalkoxy, nitro, cyano, (C_1-C_4)acyl, amino, (C_1-C_4)alkylamino, and di(C_1-C_4)alkylamino;

Y^1, Y^2, Y^3 and Y^4 are each independently members selected from the group consisting of N and C-R^2, wherein R^2 is a member selected from the group consisting of H, halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)haloalkyl, (C_1-C_4)haloalkoxy, nitro, cyano, (C_1-C_4)acyl, amino, (C_1-C_4)alkylamino, and di(C_1-C_4)alkylamino;

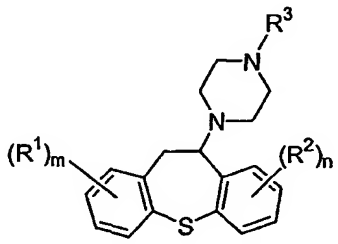
Z^1 is a divalent moiety selected from the group consisting of (C_1-C_3)alkylene;

Z^2 is a divalent moiety selected from the group consisting of –O-, -S- and -N(R^3)- wherein R^3 is a member selected from the group consisting of H, halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)haloalkyl, (C_1-C_4)haloalkoxy, nitro, cyano, (C_1-C_4)acyl, amino, (C_1-C_4)alkylamino, and di(C_1-C_4)alkylamino; and

N^{Het} is a substituted or unsubstituted 4-, 5-, 6-, or 7-membered nitrogen heterocycle.

23. A composition in accordance with claim 22, wherein X^1, X^3, X^4, Y^1, Y^2, Y^3 and Y^4 are all CH; Z^2 is –S-, and N^{Het} is a substituted 6-membered nitrogen heterocycle.

24. A composition in accordance with claim 22, wherein said compound has the formula:



wherein

the subscripts m and n are independently integers of from 0 to 3;

R^1 and R^2 are substituents independently selected from the group consisting of halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkylthio, (C_1-C_4)haloalkyl, (C_1-C_4)haloalkoxy, nitro, cyano, (C_1-C_4)acyl, amino, (C_1-C_4)alkylamino, and di(C_1-C_4)alkylamino; and

R^3 is a substituent selected from the group consisting of (C_1-C_4)alkyl, (C_1-C_4)haloalkyl and (C_1-C_4)acyl.

25. A composition in accordance with claim 24, wherein m is 0 and n is 1.

26. A composition in accordance with claim 24, wherein m is 0, n is 1 and R^2 is selected from the group consisting of halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)haloalkyl and (C_1-C_4)alkylthio.

27. A composition in accordance with claim 24, wherein m is 0, n is 1 and R^2 is selected from the group consisting of halogen and (C_1-C_4)alkylthio.

28. A composition in accordance with claim 24, wherein said compound is selected from the group consisting of methiothepin, octoclothepin and pharmaceutically acceptable salts thereof.

FIGURE 1

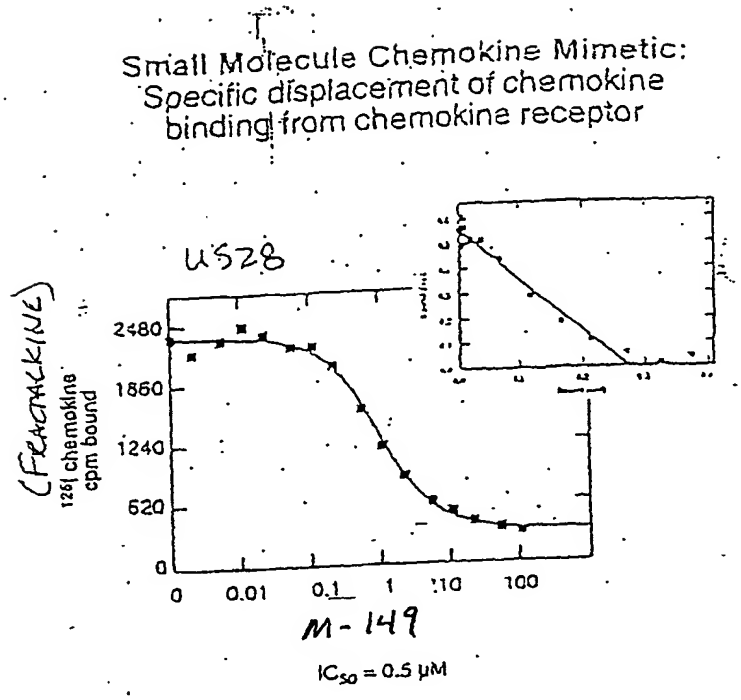
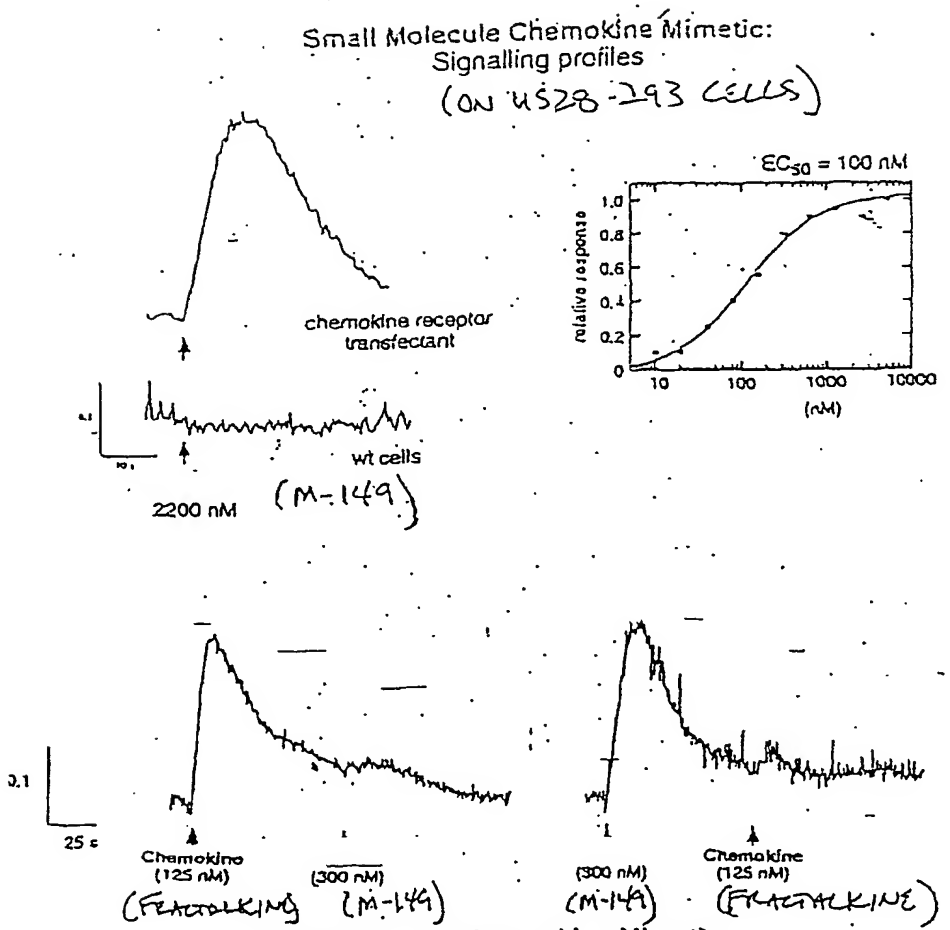

Small Molecule Chemokine Mimetic:
Specific displacement of chemokine
binding from chemokine receptor
US28
(FRACTALKINE)
125I chemokine
cpm bound
2480
1860
1240
620
0
0
0.01
0.1
1
10
100
M-149
IC50 = 0.5 µM

FIGURE 2


Small Molecule Chemokine Mimetic:
Signalling profiles
(ON U528-293 CELLS)
chemokine receptor transfectant
wt cells
2200 nM
(M-149)
EC50 = 100 nM
relative response
1.0
0.8
0.6
0.4
0.2
0
10
100
1000
10000
(nM)
0.1
25 s
Chemokine (125 nM)
(300 nM)
(FRACTALKINE)
(M-149)
(300 nM)
Chemokine (125 nM)
(M-149)
(FRACTALKINE)
Small Molecule Chemokine Mimetic:
Cross desensitization between small molecule and chemokine ligand

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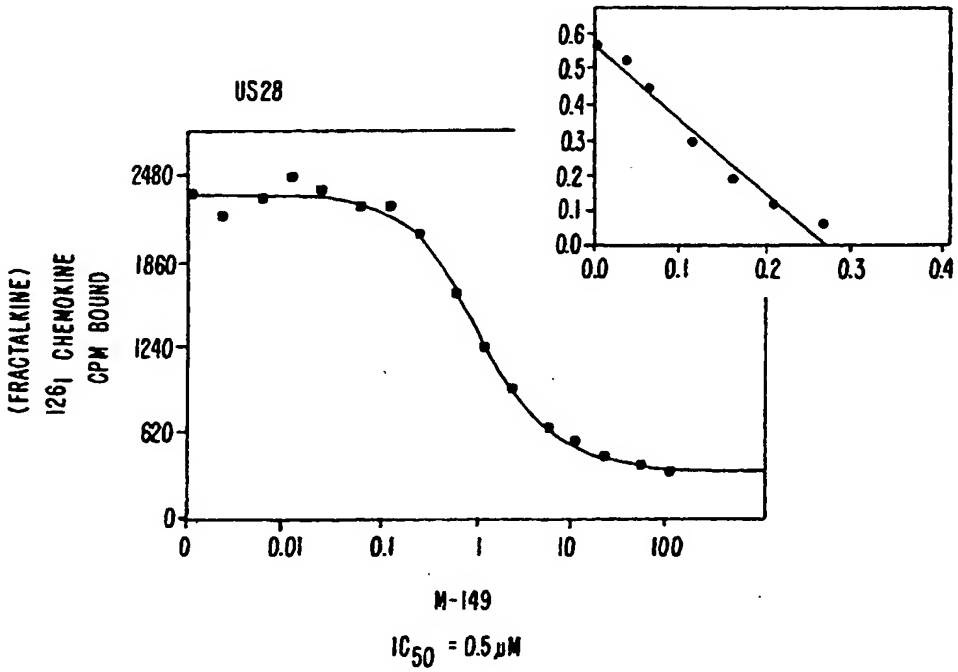
[Continued on next page]

(54) Title: MODULATORS OF US28



(57) Abstract: Assays, compositions and methods of treatment are provided for modulating the binding of chemokines to US28 on the surface of cells.

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